

EVALUATION OF TWO BIOASSAY METHODS FOR DETERMINING TOXICITY
OF SELECTED INSECTICIDES TO SWEETPOTATO WHITEFLY,
Bemisia tabaci (Gennadius)

By

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A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE
OF DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

1991

To my dear wife Melba

ACKNOWLEDGEMENTS

I wish to express my sincere gratitude to Dr. Gary L. Leibee, my adviser, for his guidance, criticism, patience and friendship. His constant encouragement and support throughout this research made my work more meaningful and brighter when I needed it the most, and for that I am very thankful.

My gratitude is extended to Dr. J. L. Nation, advisory chairman of my committee, for his constructive criticism throughout my studies, and his valuable encouragement and support during difficult times. My thanks are extended to Dr. S. J. Yu and Dr. G. J. Hochmuth for participating on the advisory committee and for the time and professional experience provided to improve the quality of this research.

Sincere thanks go to Dr. L. Osborne, Dr. D. J. Schuster, Dr. J. E. Peña, Dr. P. Stansly and Dr. S. J. Locascio for supplying materials for my research as well as for their interest in this study.

I wish to express my appreciation to the personnel of the Central Florida Research and Education Center in Sanford and the Department of Entomology and Nematology in Gainesville. Especial thanks go to Kenneth Savage for his assistance during my research as well as to Sue Wilson, Laura Seckbach, Charlene Di Nicola, Carolyn Pickle, Myrna Litchfield and Sheila

Eldridge for their patience and willingness to assist me during the development of my studies. I am grateful for the statistical assistance provided by A. Raychaudhuri, S. Kundu, and Dr. R. Littell from the Statistics Department.

I wish to thank Dr. D. Borovsky for giving me the opportunity to begin my doctoral studies in this country and to Dr. J. Maruniak for his help and assistance in his laboratory. I also extend my sincere gratitude to Dr. D. Young at the Medical Entomology Department for his kindness and friendly help upon arrival at the university.

Financial support for this research was provided in the form of an assistantship funded by Dr. Gary L. Leibee.

I would also like to extend my gratitude to Hernando Moreno for his support and encouragement and to Bob Gardner for providing advice with his computer skills. Special appreciation goes to Drs. Edward and Nadja Golding for their love, support and friendship, and to Mike and Lisa Sever from Gainesville for their spiritual encouragement and endless love toward my wife and me. Special thanks go to my wife Melba for helping me type this thesis, but more than that, for her patience and unconditional love. Her companionship and support throughout my studies made my hardships seem insignificant.

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Abstract of Dissertation Presented to the Graduate School of
the University of Florida in Partial Fulfillment
of the Requirements for the Degree of Doctor of Philosophy

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August 1991

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Leaf residue and sticky tape bioassay methods were used to determine toxicity levels of selected insecticides to the sweetpotato whitefly (SPWF), Bemisia tabaci (Gennadius). Dose-mortality response to endosulfan, chlorpyrifos, fenvalerate, and bifenthrin to SPWF adults was highly reproducible for both bioassays. The sticky tape method did not work well for abamectin. This was probably due to a rapid photodegradation of abamectin on the residual tape following application. The leaf residue method was more representative of the situation in the field. The sticky tape was more convenient for field application and the tape is easily adaptable to different situations, hence facilitating different sampling schemes in the field. The major drawback with the sticky tape was that excessive mortality (apparently

due to desiccation) occurred unless the adults were held in a humid environment. Insecticide persistence on sticky tapes was examined. Results showed that all the insecticides studied maintained their toxic effect at the end of the fifteenth day of the study.

The LC_{50} values for endosulfan, chlorpyrifos, fenvalerate, bifenthrin, and abamectin found with the leaf residue bioassay were 0.124, 0.575, 0.153, 0.104, and 0.0002 mg [AI]/ml, respectively. The LC_{50} values for the same insecticides with the sticky tape bioassay were 0.223, 0.840, 0.874, 1.460, and 0.0072 mg [AI]/ml. There was a significant effect due to size and age of the SPWF adults and their interaction on mortality. Large adults two or three days old were used as standard for the bioassays.

The sticky tape bioassay was used to evaluate five populations of the SPWF from Florida for insecticide resistance to commercial insecticides. Low susceptibility responses to endosulfan from the Immokalee strain and to fenvalerate from the Gainesville strain were found when compared with the other strains. The low resistance ratios for these populations ($RR = 1.7$ for endosulfan and $RR = 1.6$ for fenvalerate, respectively) do not imply that resistance will not increase, but rather that it has not occurred to a significant degree in the adult stage of the SPWF. These results need to be considered for monitoring programs in insecticide resistance in the SPWF in Florida.

CHAPTER 1

INTRODUCTION

The sweetpotato whitefly (SPWF), Bemisia tabaci Gennadius, is a polyphagous pest of many commercial and agronomic crops throughout the world. During the past two decades, the SPWF became a serious pest attacking at least 500 species of plants worldwide (Greathead 1986). Early outbreaks were reported in cotton in the 1930s in India (Husain and Trehan 1933) and the insect was described mostly as the tobacco whitefly (Mound and Halsey 1978). Subsequent outbreaks of SPWF on cotton occurred in other tropical and subtropical areas, such as in Israel, Sudan, Turkey, Zimbabwe, Central America and the United States (Mound 1963, Gamez 1971, Russell 1975, Gerling et al. 1980, Duffus and Flock 1982, Musuna 1983, Dittrich et al. 1985).

The SPWF causes direct damage by sucking sap from the plant, and indirectly by vectoring several phytopathogenic virus diseases resulting in heavy crop yield losses (Dittrich et al. 1985, Brunt 1986, Cohen and Berlinger 1986). Indirect damage inflicted by the transmission of plant pathogens by the SPWF on several crops in California and Arizona has been assessed at one hundred million dollars (Duffus and Flock 1982). In addition, the SPWF interferes with the normal

development and quality of the crop by leaving abundant deposits of honeydew on leaves that allow sooty molds to grow.

In the mid-1980s the SPWF became an important agricultural pest of many ornamental and vegetable crops in Florida (Hamon and Salguero 1987, Schuster and Price 1987). Infestations of SPWF have already become a great menace in Florida's ornamental industry because of the cosmetic damage they inflict on crops. The appearance and quality of ornamental plants are important factors in the marketing of poinsettias (Black et al. 1984), hibiscus, chrysanthemum, gerbera daises, and other bedding plants. Among the vegetable crops reported to be attacked by the SPWF in Florida are eggplant, pepper, cucumber, melon, squash, and tomato (Schuster and Price 1987). Heavy infestations of the SPWF on Florida tomatoes were first reported in 1987 by Schuster et al. (1989), who described losses of \$15 million by the spring of 1988. According to Pohronezny et al. (1986), tomato production is economically the most important vegetable crop grown in Florida, where almost 98% of tomatoes are grown for fresh market.

In 1989, irregular ripening was the most important damage on tomatoes associated with the SPWF (Stansly and Schuster 1990). The agent responsible for this disorder is unknown (Schuster et al. 1989). SPWF is a menace to Florida's agriculture for its ability to vector several infectious diseases as reported for lettuce, melons, cucurbits, and

sugarbeets in Arizona and California (Duffus and Flock 1982). The relationship of SPWF to transmission of virus diseases to Florida tomatoes has recently become a major focus among researchers after several viral symptoms were reported on tomatoes during the fall of 1989 (Vavrina 1990). Most infected plants exhibited intervenal mottling of new leaves, upward curling and distortion of leaflets, downward arching of petioles and stunting (Stansly and Schuster 1990). The causal agent was determined to be a geminivirus (Hiebert 1990, Brown 1990). Control of SPWF in attempting to prevent virus infection has resulted in a great increase in usage of insecticides in tomato production. The problem of insecticide resistance as a consequence of this intensified chemical control represents a real threat to Florida agriculture, especially considering the SPWF's propensity for developing resistance to insecticides (Prabhaker et al. 1985).

Various control programs for the SPWF have been implemented worldwide. They include release of natural enemies (Gerling 1986, Osborne et al. 1990), cultural control (Schuster et al. 1989), host plant resistance (Berlinger 1986), and insecticides (Price 1987). The most controversial is the use of insecticides because they are blamed for the elevation of the SPWF from a secondary to a primary pest. When outbreaks of SPWF occur, insecticides are still considered the most effective control method. However, there are two major problems with insecticides. One is the

phytotoxicity of some insecticides in a susceptible crop such as poinsettia, (Price et al. 1987) and the other is the development of insecticide resistance (Prabhaker et al. 1985). Although insecticide resistance in the SPWF has not been reported to be serious in Florida, the availability of practical techniques to monitor levels of insecticide susceptibility and data on baseline toxicity of insecticides to the SPWF would be extremely valuable tools for characterizing suspected episodes of insecticide resistance in the future. If an insecticide resistance episode with the SPWF is confirmed, implementation of appropriate resistance management strategies could be made in a timely manner.

Although some Integrated Pest Management (IPM) strategies have been used to control the SPWF (Berlinger 1986, Gerling 1986, Schuster et al. 1987), managing insecticide resistance in whiteflies is rather a new study. Since SPWF became resistant to several insecticides (Prabhaker et al. 1985), more studies are needed to develop practical methods for monitoring insecticide resistance and to determine baseline toxicity data for Florida populations of SPWF. This study was conducted for the following purposes:

1. To evaluate two techniques for determining the toxicity of insecticides important for the control of SPWF in Florida.
2. To use these techniques to characterize the response of a susceptible strain of SPWF to the insecticides.

3. To use these techniques and baseline data to examine several Florida populations of SPWF from commercial situations for insecticide resistance.

CHAPTER 2

LITERATURE REVIEW

The Sweetpotato Whitefly (SPWF)

Taxonomy

The sweetpotato whitefly, Bemisia tabaci Gennadius, belongs to the subfamily Aleyrodinae, family Aleyrodidae, and superfamily Aleyrodoidea. Some taxonomists place it in the suborder Homoptera, order Hemiptera (Woodward et al. 1970), while others put it in the suborder Sternorrhyncha, order Homoptera (Borror et al. 1976).

Classification of the Aleyrodids at the family and subfamilial level is based on adult morphology, but genera and species are best defined on the structure of the fourth nymphal instar, the so-called "pupal case" stage (Mound and Halsey 1978). Aleyrodid pupal cases provide many characters which are used to assist in the identification of species (Martin 1987), and most of these are defined and discussed by Russell (1948). However, Russell (1948) found a variability in the structure of the pupal case in some collections of Trialeurodes vaporariorum (Westwood) that could be correlated with the structure of the host leaf. Later Mound (1963) reported that the appearance of the pupal cases of B. tabaci depends on the form of the host plant cuticle on which they

develop. This host-correlated variation led to a number of former "species" of Bemisia being synonymized with B. tabaci, such as B. gossypiperda (Misra and Lamba) from India and Pakistan, B. goldingi (Corbett) and B. rhodesidensis from Nigeria (Mound 1963).

B. tabaci was first described as Aleurodes tabaci by Gennadius (1889) from tobacco, Nicotiana sp. in Greece. The most common names of B. tabaci referred to in the literature are tobacco whitefly, cotton whitefly, and sweetpotato whitefly (Lopez-Avila 1986a). Aleyrodid pupal cases have provided many characters used in the identification of whitefly species and keys for their identification have been defined by Russell (1975), Mound and Halsey (1978), and Martin (1987).

Biology

The sweetpotato whitefly is a polyphagous pest in tropical and subtropical regions. The adults are small (about 1 mm. long), soft, and completely white insects due to the deposition of 'wax' particles that cover the exterior surface on their bodies and wings. It is from this distinguishing wax feature that the family takes its name (Aleyro [Greek] means flour or meal) (Byrne and Hadley 1988). Immature stages look like scale insects and feed on the abaxial leaf surface of many agronomic and ornamental plants of economic importance. These immature stages are usually divided into three nymphal

stages (EL-Helay et al. 1971) and the so called "pupal case" stage (Mound 1963).

Eggs of the SPWF are oval in shape and have a short stalk which serves as an attachment to the host plant. Before hatching, eggs turn from whitish-yellow color to light brown (Lopez-Avila 1986a). Newly hatched nymphs have well developed legs, functional antennae and are usually called "crawlers" because they crawl over the leaf surface in order to find a suitable place to insert threadlike mouth parts to feed. Once they start to feed, the crawlers usually do not move, and they transform successively into the second and third legless nymphal instars. The fourth instar or pupal stage is elliptical in shape with the cephalic region semicircular. The dorsal surface is convex and the thoracic and abdominal segments are apparent. It is 0.700 mm long and 0.376 ± 0.02 mm broad at the mesothorax (Lopez-Avila 1986a). The duration of the pupal stage varies depending on the temperature. Butler et al. (1983) found that at high fluctuating temperatures of 26.7°C to 43.3°C, 74% of 291 pupae delayed their development on cotton leaves. When pupae were transferred to 25°C they immediately continued their development into the adult stage. Emergence of adults occurs from a slit in the top of the pupa and according to most researchers the period of maximal emergence falls between the hours of 0800 and 1200 (Husain and Trehan 1933, Butler et al. 1983). Adult longevity varies according to climate and

temperature conditions. For instance, Butler et al. (1983) found that at 26.7°C and 32.2°C males lived an average of 7.6 and 11.7 days, and females lived an average of 8.0 and 10.4 days, respectively. Lopez-Avila (1986a) found that under standard laboratory conditions (25°C, 60% RH, L:D 16:8) the longevity varied from 5 to 15 days (mean 8.66) for males and 5 to 32 days (mean 19.75) for females. Females feed for several hours or even for one or more days before laying their first eggs.

Since climate and host plant conditions can considerably affect reproduction of the SPWF (Gerling and Horowitz 1986), different life cycle results have been reported. El-Helay et al. (1971) in studies conducted on sweetpotato, Ipomoea batatas L., reported a total life cycle of SPWF of 11.4 ± 0.3 days at a temperature of $31.01 \pm 1.0^\circ\text{C}$ and of 15.9 ± 0.3 days at $25.4 \pm 0.4^\circ\text{C}$. Butler et al. (1983) in studies on cotton, Gossypium hirsutum L., reported that development of SPWF from egg to adult took 23.6 ± 1.4 days at 25°C and 16.1 ± 1.6 days at 30°C. In contrast, Husain and Trehan (1933) found that completion of the life cycle on cotton varied from 14 to 107 days, but generations occurred each 14 to 21 days during April to September when average temperatures were of 37°C to 29°C. Studies conducted by Coudriet et al. (1985) on the development of the SPWF on 17 hosts at $26.7 \pm 1^\circ\text{C}$ greenhouse-controlled temperature found that SPWF life cycle varied greatly from 18.6 ± 1.1 days on sweetpotato to 29.8 ± 2.2 days on carrots.

Price et al. (1987) reported that SPWF developed in about 23 days on poinsettias grown at summer temperatures (about 27°C-35°C).

Effects of the temperature and leaf age upon age-specific fecundity and relative oviposition rate of SPWF has been reported by Von Arx et al. (1983). Sharaf and Batta (1985) stated that the fecundity of SPWF was 76.0 eggs and 56.4 eggs at 25°C and 14°C, respectively. The preoviposition period found was 3.6 and 4.9 days for the two temperatures. The pronounced influence of insecticidal treatments on fecundity has been discussed by Dittrich et al. (1985). They report that repeated and frequent insecticide applications on cotton to control populations of SPWF have created an insecticide resistance and high fecundity in SPWF females.

Females of the SPWF have the ability to reproduce in the absence of fertilization (Arrhenotoky), thus virgin females initiate field populations until emergence of their male progeny (Husain and Trehan 1933, Gerling and Horowitz 1986). Sharaf and Batta (1985) found that a decrease in temperature from 25°C to 15°C caused an increase in the number of adult females. The sex ratios were 1:1.8 and 1:3.1 (male:female), respectively. Lopez-Avila (1986a) found a sex ratio of 1:2.15 (male:female) for the SPWF at 25°C during more than 10 generations.

Population Ecology

Sampling programs are an essential requirement in any study to determine the population ecology of a pest. It is necessary to distinguish the sampling method to be used in order to assess correctly the incidence of the pest and its natural enemies, as well as to estimate its population dynamics.

Several sampling methods and different life stages have been considered in studies on the population ecology of B. tabaci (Gerling et al. 1980, Von Arx et al. 1984, Horowitz 1986, Meyerdirk et al. 1986). According to Ohnnesorge and Rapp (1986), population estimates of SPWF can be obtained by monitoring adults and larval stages separately. Monitoring the adult population is done by visual counts or by catches with yellow sticky traps or suction traps. Sampling of third and fourth instar larvae estimate the absolute population density and is usually made by counting all the larvae present on a leaf. Sampling methods most used on the SPWF adult include sticky traps, suction samplers, and counts of whiteflies per plant unit (Cock 1986).

Yellow sticky traps consist of yellow plastic sheets, plates, or Petri dishes kept in position by poles or fixed to the ground by small stakes (Ohnnesorge and Rapp 1986). They are coated by a sticky substance such as grease, tanglefoot (Tanglefoot Co), or another dilute adhesive (Berlinger 1980). When whiteflies are counted and the adhesive is removed by a

detergent or solvent the trap can be recoated and used again. Yellow sticky traps have often been used to monitor SPWF populations (Sharaf 1982, Butler and Wilson 1984, Byrne et al. 1986, Musuna 1986, Schuster et al. 1989). Traps of various designs and placement (Sharaf 1982, Byrne et al. 1986) have been installed in different crops to study the dispersal flight pattern of SPWF (Gerling and Horowitz 1984, Meyerdirk et al. 1986).

Examination of trap catches has helped researchers to determine patterns of flight and time of adult emergence. Gerling and Horowitz (1984) reported that catches of SPWF adults on yellow sticky traps in cotton fields were greater on traps placed horizontally rather than vertically. The largest catches were obtained at ground level despite the fact that the height at which most whiteflies existed in the air exceeded 2 m. Similar results were found by Byrne et al. (1986) in which traps placed at ground level caught significantly more insects than traps of the same design placed at greater heights. They also reported that SPWF and the bandedwinged whitefly, Trialeurodes abutilonea (Haldeman), leave their pupal cases at the same time, within an hour of the onset of daylight. Determinations of the time of emergence and readiness of whiteflies to take flight are important in indicating when to place sticky traps for monitoring whitefly populations, as has been done

similarly with the greenhouse whitefly, Trialeurodes vaporariorum (Byrne et al. 1986, Gillespie and Quiring 1987), the bandedwing whitefly, T. abutilonea (Lambert et al. 1982) and B. tabaci (Gerling and Horowitz 1984, Byrne et al. 1986). Timing of chemical application as well as the timing of introduction of natural enemies are also dependent upon monitoring (Yano 1987). For instance, predators and parasitoids usually appear in crops at a certain time of season, especially when whitefly populations are high and pesticide application is low (Johnson et al. 1982). Natwick and Zalom (1984) found lower populations of SPWF during the fall because of the high parasitism by Eretmocerus haldemani, which reached levels >70% in cotton fields. In contrast, Toscano et al. (1985) reported that SPWF population growth on cotton in California was rapid, apparently because pyrethroid-based insecticides used in the area reduced the number of parasitoids. The usefulness of yellow sticky traps to determine the time of introduction of the parasitic wasp, Encarsia formosa, on tomato crops was reported by Yano (1987). He found low levels of whitefly infestations when the parasites were introduced after monitoring by yellow sticky traps.

The use of suction samplers such as D-vacs and modified vacuum cleaners is another technique for sampling SPWF (Gerling and Horowitz 1984). Suction traps consist basically of a strong air current that aspirates whitefly adults from

their host into a collection bag (Ohnesorge and Rapp 1986). Suction traps usually allow large areas to be sampled within a short time.

Sampling of SPWF on a per plant basis seems to be the recommended tactic, though it is very time consuming due to the varying distribution of whiteflies on the plants. According to Ohnesorge et al. (1980), adults and eggs tend to occur mostly on young leaves because females prefer these leaves as oviposition sites. The sessile stages mature with the leaves on which they hatched. Pupae are found on older leaves and leaves that are decaying. This distribution of SPWF on the plant therefore requires one to sample adults and nymphs separately (Ohnesorge and Rapp 1986, Horowitz 1986). Direct counting of SPWF adults on plants is preferably done in the morning when adults are least mobile (Horowitz 1986). Cock (1986) recommends that nymphs should be sampled on a leaf or defined portion of the leaf basis as result of the non-random distribution on the plants (Ru-Mei 1982). Other models for distribution samplings of B. tabaci have been regarded as numerical and sequential sampling with a given precision level (Von Arx et al. 1984), and stratified random sampling as proposed by Ohnesorge and Rapp (1986). Documentation of factors that affect or influence SPWF populations such as immigration, emigration, climate, and natural enemies has been comprehensively reviewed by Cock (1986).

Host Plants

The SPWF is a worldwide pest that attacks a wide variety of host plants. Host plants of this pest belong to not less than 74 families in which 506 plant species have been reported to be susceptible either to adult or nymphal feeding (Greathead 1986). Plant families more often reported are Leguminosae, Solanaceae, Malvaceae, Euphorbiaceae, Cucurbitaceae, Compositae, Labiatae, Cruciferae, Rosaceae, and Moraceae (Greathead 1986).

Many Florida greenhouse growers have reported severe attacks of SPWF on ornamental and vegetable crops (Alderman 1987). Poinsettias, hibiscus, chrysanthemum, squash, melon, pepper, and tomato are among the most recorded hosts for SPWF.

It is assumed that whiteflies, in general, are attracted to their host plants from a distance by visual (color) cues and that their host acceptance is determined by contact cues, touch, and taste (Berlinger 1986). Host acceptance by the insect seems to be finalized by piercing and probing the plant with its mouth parts. Berlinger (1986) reported that host plant selection is affected mainly by the following features: (1) the external physical characteristics of the leaf surface, e.g. hairiness vs. glabrousness, sticky glandular trichomes, leaf shape and probably microclimate as a result of foliage density, and (2) the internal, chemical characteristics of the leaf, e.g. pH of leaf sap. On cotton, for instance, leaf-hair density and leaf shape (okra/super okra) play a crucial role

in whitefly host-plant relationship. Highly pubescent cotton cultivars bear larger populations of SPWF than do glabrous types (Mound 1965).

Coudriet et al. (1985) determined the host-range and developmental rate of SPWF on several commercially grown crops in California. They reported that the SPWF required 30% less time to complete its life cycle on squash, eggplant, cucumber, and lettuce than on carrots and broccoli. On weed hosts, Coudriet et al. (1986) reported that the type of host to which B. tabaci was confined had an impact on its population dynamics. The list of weed hosts is long and many of them are found in Florida, such as spurge (Euphorbia sp.), nightshade (Solanum sp.), morning glory (Ipomoea sp.), hairy indigo (Indigofera sp.) and primrose willow (Primula sp.) (Schuster et al. 1989).

The sweetpotato whitefly also attacks primary nutritional crops such as cassava (Manihot esculenta Crantz), soybean (Glycine max), and common bean (Phaseolus vulgaris) in tropical areas of Africa (Robertson 1987) and Latin American (CIAT 1986) countries. For instance, in Zimbabwe whiteflies have become a potential threat to cotton crop production and also has been found in cassava and sunflower (Musuna 1983).

Economic Importance

Bemisia tabaci Gennadius, is a well known economical pest throughout tropical and subtropical areas, not only causing direct damage to a large number of crops but also serving as

a vector of virus-transmitted diseases. Outbreaks of SPWF were first reported in cotton fields in India between the 1920s and 1930s (Husain and Trehan 1933). It then appeared in other areas such as in Sudan and Iran by the 1950s (Joyce 1955), Central America (1961) (Kraemer 1966), Brazil (1968) (Costa 1976), Turkey (1974) (Habibi 1975), and Israel (1976) (Gerling et al. 1980).

In the United States, SPWF was first collected from cotton in Arizona in 1926 and California in 1928 (Russell 1975). Although the SPWF had previously been reported as a sporadic pest (Gerling 1967), it was not until 1981 that the SPWF was considered a primary pest. Unexpected high populations of SPWF in cotton and vegetable crops began to cause enormous damage in the southwestern desert valleys extending through southern California and Arizona (Meyerdirk et al. 1986). SPWF transmitted several infectious disease agents to cotton, vegetable, and sugarbeet crops causing losses in excess of \$100 million (Duffus and Flock 1982).

Outbreaks of SPWF were also recorded in northern Panama and southwestern Sao Paulo (Brazil) on soybean, common bean, and cotton in the late 1972 and early 1973 (Kogan and Turnipseed 1987). These crops were subject to direct damage by high populations of SPWF as well as to several phytopathogenic virus diseases vectored by SPWF. In Costa Rica, for instance, the main damage that SPWF causes on tomatoes is the transmission of a geminivirus, which probably

is the causal agent of tomato yellow mosaic (TYM) (Rosset et al. 1990). In the last decade, B. tabaci became an important pest on bean crops (e.g. Phaseolus vulgaris) in Central and South American countries, such as in Salvador, Guatemala, Nicaragua, Costa Rica, Panama, Colombia, Argentina, and Brazil (CIAT 1986). SPWF caused great losses in bean production in these countries, with the transmission of the well-known bean golden mosaic virus (BGMV) (Gamez 1971).

Severe economic damage by SPWF occurs directly and indirectly. Direct damage is caused by sucking sap from the plant creating plant injury such as chlorotic spots at feeding sites on leaf surfaces, leaf shedding, and reducing growth rate in some crops. Their feeding often causes heavy yield losses. Indirect damage occurs by vectoring several transmitted diseases causing lower crop yields. Besides transmission of plant pathogens, SPWF causes decrease of crop quality and fiber as a result of the accumulation of honeydew, which reduces photosynthesis and affects other physiological processes in host plants (Lopez-Avila 1986c).

The most important virus-transmitted diseases caused by SPWF are the cotton leaf curl (CLC) (Duffus and Flock 1982), lettuce infectious yellows (LIY) (Duffus et al. 1982), cucurbit leaf curl (CLC) (Dodds et al. 1984), tomato yellow leaf curl (TYLC) (Cohen and Berlinger 1986), and tomato golden mosaic (TGM) (Costa 1976). Newly hatched nymphs (crawlers)

and adults of SPWF have the ability to spread the viral plant diseases by picking up virus particles when they feed on infected plants. When adults move and subsequently feed on susceptible healthy plants, they spread the viral disease.

The economic losses caused by the SPWF on cotton and vegetables since 1981 range in the millions of dollars (Toscano et al. 1985). Nazer and Sharaf (1985) state that yield losses in the tomato industry in the Jordan Valley (Middle East) reach as much as 63% during the fall season, as a consequence of the tomato yellow leaf curl (TYLC) disease transmitted by the SPWF. Bemisia tabaci has also caused severe economic problems in African countries with the incidence of other transmitted diseases in main crop fields. Robertson (1987) has reviewed research studies conducted in tropical Africa on the epidemiology of the African cassava mosaic virus (ACMV) transmitted by the SPWF and the ecology of this vector on the coast of Kenya.

In Florida, it has been estimated that SPWF causes annual losses of at least \$25 million to tomato producers due to increased control costs as well as direct fruit yield losses (Schuster et al. 1989). According to Ball (1987), in 1986 the SPWF was ranked as the most important pest of Florida's \$8 to \$10 million poinsettia industry. In 1988, a severe outbreak of SPWF in southern Florida was believed to be the cause of irregular ripening in tomatoes which threatened to cause \$200 million in damage in Broward, Collier, Dade, and Palm Beach

counties (Woods 1988). The SPWF was also related to the induction of silvering of squash in the same counties (Cantliffe 1989). During the fall growing season of 1989, tomato and pepper crops in Florida were affected for the first time by viral diseases presumed to be associated with unprecedented high population levels of SPWF (Brown 1990, Vavrina 1990). According to these authors, the majority of whitefly-transmitted viruses now recognized in North America are members of the Geminivirus group. These geminiviruses infect cucurbits, legumes, solanaceous plants such as tomato and pepper, malvaceous crops such as cotton, and Euphorbia species. Several tomato samples exhibiting "geminivirus" symptoms from the affected counties were processed and the virus inclusions present were confirmed to be geminivirus particle aggregates (Kring et al. 1990). These authors demonstrated that adults of SPWF collected from infected field plants transmitted geminivirus symptoms with a 70% efficiency to caged, healthy tomato plants in greenhouse experiments.

Management of the SPWF

Cultural Control

Cultural and mechanical control measures have been the oldest methods used to control insects. These methods use direct or indirect measures to (1) destroy the insect, (2) modify the environment or planting conditions to make it undesirable for the insect, or (3) prevent or disrupt the normal life processes of the insect (Splittstoesser 1984).

Although some of these tactics require considerable time and effort, practicing appropriate cultural control procedures needs consideration in any IPM program, especially when they can have an impact on the behavior and population dynamics of both the pest and its natural enemies. Herzog and Funderburk (1986) describe "cultural control" as a cultural practice that has detrimental impact on insects and is needed in preventive programs. Most of the control tactics are usually the farmer's first management procedure. Some of those control tactics that have been used on different crops include resistant or tolerant plant cultivars, tillage practices, crop rotation, sanitation requirements, and varying of planting dates. Changes in cultural practices such as switches from multiple to single cropping, variation in planting date, wide to narrow row spacing and changes in pesticide patterns or usage in a crop alter the ecological conditions of the plant as a host (Herzog and Funderburk 1986).

Management of the SPWF with cultural control needs to be exploited where possible to minimize the use of chemical control. Very few reports have described cultural control tactics to apply against whiteflies and their whitefly-borne viruses. Cock (1986) reviews some cultural methodologies used in different areas against the SPWF, such as destruction of alternate hosts, use of straw mulch, date of sowing, isolation of crops and trap crops. Some of them, however, have not been followed up and are not used.

Some of the cultural control measurements of SPWF on ornamental plants such as poinsettias reported by Price et al. (1987) include (1) exclusion of a crop (at least 1 week) that had been infected previously by whiteflies to starve adults from the previous crop, (2) removal of weeds that serve as alternative hosts from within and around the poinsettia production area, (3) destruction of weeds and other infested plants by burning and bagging, (4) purchase of only poinsettia cuttings that are free of whiteflies and restriction of sales of infested whitefly crops, and (5) avoiding use of yellow clothing and equipment because whiteflies are attracted to yellow colors.

Cultural control of the SPWF on tomato crops in Florida (Schuster et al. 1989), include the followings steps: 1) location of the plant production facility away from infested areas if possible and screening to exclude invading SPWF adults, 2) isolation of fields well away from double cropped or infested crops, 3) preparation of the land at least a month before transplanting and, 4) destruction of weeds and other volunteer crop areas within the field perimeter. The use of plastic mulches helps to repel alighting aphids and to delay the appearance of virus that they may transmit. Post-harvest activities (destruction of crops soon after harvest, herbicide and insecticide application in the field to kill remaining SPWF immature and adults), and host plant resistance are also suggested. Another cultural strategy requires that

tomato plants go into the ground, as much as possible, free of whiteflies and virus. The latter needs serious consideration because Florida tomatoes have been reported to be infested by geminivirus since the fall of 1989 (Kring et al. 1990, Vavrina 1990).

In California, complete exclusion of SPWF from squash plants at seedling emergence until initial fruiting has proved effective in preventing virus disease infection for a critical period during production (Natwick and Durazo 1985). This has been possible through the use of spun-bonded polyester (SBP) material as a floating row cover. The floating row cover can be sealed at the sides and ends of the beds with soil and can be placed directly over plants whether they were started as transplants or direct seeded. Natwick and Durazo (1985) compared the effectiveness of insecticides and SBP row covers in controlling the SPWF and thus the suppression of virus disease. SBP floating row covers were placed over four beds of zucchini after planting and before irrigation. Several insecticides were applied to zucchini seedlings on non-SBP-covered beds when the plants were at the first and second leaf stage and again when at the fourth to six leaf stage. The objective of the insecticide treatment was to control SPWF adults migrating into squash planting. The row cover and untreated check plots were not sprayed with insecticide. Counts of SPWF adults on leaves of squash seedlings in both the insecticide-treated and check plot (non-cover) were very

high, ranging from 94 to 911 and from 556 to 3,820 per plant, respectively. Endosulfan or combination of other insecticides with endosulfan provided the best control. Floating row covers excluded SPWF from the zucchini plants. The virus disease ratings were based on the number of zucchini plants with lettuce infectious yellows or squash leaf curl virus symptoms per 10 plants examined in each of four replicates. Virus disease symptoms were apparent in 75 to 100% of the zucchini plants four days after the second insecticide application (only 12 days after the seedling emerged). Under floating row covers, virus disease symptoms did not appear until the plants were large (18 in tall) and were flowering.

Another means of delaying the onset of virus symptoms with cultural control is with the use of colored mulches in the fields, which apparently repel whiteflies. Dr. J.B. Kring (IPM Practitioner 1990) from the GCREC, UF/IFAS, Bradenton, FL, experimented with repellent mulches (clear, aluminum and various colors) for SPWF control. He found that protection apparently arises from the amount of ultraviolet light reflected. These mulches influence plant growth through influence on soil and leaf temperature by selectively transmitting certain wavelength of light and reflecting others (Stansly and Schuster 1990). Kring et al. (1990) found the greatest number of SPWF trapped on red-colored-mulch protected tomatoes. With orange and yellow, SPWF adults were attracted to the mulch and not to the plant. On the other hand,

aluminum seemed to repel the insects from the mulch surface and the plants. It also delayed viral disease symptoms one to two weeks more than white mulch.

In African countries, sanitation is one of the main tactics for control of African cassava mosaic virus (ACMV) disseminated by the SPWF and by man through infected cassava stem cuttings. The use of cultural techniques such as sanitation, which includes selecting and planting healthy cuttings, could rapidly decrease the impact of ACMV and improve cassava production in Africa (Fauquet and Fargette 1990).

Host Plant Resistance

Host plant resistance (HPR) to pests has been discovered in many crops in various parts of the world. Plant resistance to insects was used as a primary method of pest insect control long before the advent of synthetic organic insecticides (Adkinson and Dyck 1980). Only a few pests have been controlled for many years by use of resistant varieties alone. Insects in which host plant resistance has been successful often have been those with a high host specificity, such as in the case of aphids and scales (Painter 1951).

Berlinger (1986) identified host plant resistance (HPR) to insect pests as "any reduction in population growth rate of the pest population, influenced by the host plant." According to Berlinger, breeding for resistance is based on the concept that wild plants first defend themselves against herbivores by

means of repellent or toxic plant chemicals. Determination of the basis of the plant's resistance mechanisms is essential to properly use resistant cultivars in IPM programs (Wiseman 1987). The key to success for HPR lies in its incorporation into management systems involving other control measures, such as regulated planting dates, early harvesting and crop residue disposal, manipulation of alternate hosts, host-free periods, and destruction of overwintering pest insects (Adkisson and Dyck 1980).

Many institutional entomology programs throughout the world are conducting breeding programs to develop resistant varieties. For instance, in the case of soybean pest control, several HPR components are being bred to develop resistant varieties against several bean pests. These sources of resistance against bean pests and other pest complexes are being sought in world germplasm banks (CIAT, personal communication). However, breeding for resistant varieties is a long-term, complicated, and expensive task (Cock 1986).

Very few studies have been reported relative to host plant resistance to the SPWF and its relation to the transmission of viral diseases. As stated by Ohnesorge and Gerling (1986), the present knowledge of B. tabaci plant relationships is too scanty to allow for full exploitation of the resistance resources within plants. Moreover, plant cultivars are continuously being developed in cotton, vegetables, and other crops. The possible reaction of SPWF to

these cultivars and to their specific characteristics, such as secondary plant substances, leaf thickness, pubescence, etc., are unknown.

Studies of host plant resistance to the SPWF have mostly been conducted by Berlinger (1986). He states that various plant species show different levels of resistance to SPWF. For instance, completely resistant plants are not attacked at all and are considered to be immune. On resistant plants, the pest population will not reach the economic injury level (EIL) before maturity. On partially resistant plants, the EIL is reached late in the season. On susceptible plants, the EIL will be reached in the season and intensive control measures must be applied by the grower to save the yield.

Of the crops attacked by the SPWF, only resistance in cotton, Gossypium hirsutum, has been obtained to a certain degree. It seems that leaf-hair density and leaf shape play a crucial role in whitefly-host plant relationship on this crop. For instance, Mound (1965) found that highly pubescent varieties of cotton were more heavily infested by the SPWF than glabrous varieties. Modification of the cotton leaf shape also seems to be an effective means of increasing tolerance to the SPWF. Sippell et al. (1987) reports that okra and super okra leaf shape confer high degree of resistance to SPWF. Other crops, such as Lycopersicon hirsutum and L. hirsutum f. glabratum accessions have been found to be significantly resistant to SPWF (Berlinger et al.

1983) as well as to the greenhouse whitefly (de Ponti 1983). Their resistance was due partially to their glandular leaf hairs exuding a toxic compound.

Other factors such as pH in food selection, climatic conditions (light duration and intensity), plant nutrition (high nitrogen content of the leaves, soil or water salinity), and secondary metabolites in the leaves (i.e. high levels of bud gossypol) have been studied and considered as possible factors that may influence plant resistance to the SPWF (Berlinger 1986). In general, plant resistance to SPWF is due to external leaf features, hairiness vs. glabrousness in cotton, toxic exudants of the trichomes in wild tomato, or a sticky compound, probably in combination with a toxic factor in the exudate S. penellii (Berlinger 1986). However, breeding for resistance to SPWF is still in the early stages and further studies need to be conducted.

Biological Control

Several species of parasitoids and predators are potential biocontrol agents for B. tabaci. Parasitic wasps of the genera Encarsia and Eretmocerus, both of the family Aphelinidae (Hymenoptera), have been documented as effective parasitoids against SPWF (Lopez-Avila 1986b, Gerling 1986). Adult female wasps deposit their eggs in the bodies of the larvae and pupae of SPWF. The parasitic larvae feed on the body fluids of immature whiteflies. At 24°C, Encarsia formosa requires 15 days to develop from egg to adult, while

Eretmocers haldemani takes 22 days (Johnson et al. 1982). Some species of Encarsia prefer only third and fourth instars of Bemisia tabaci for oviposition while Eretmocers species prefer to lay eggs on the second and third instar of SPWF (Johnson et al. 1982, Osborne et al. 1990).

According to Hoelmer and Osborne (1990), five species of these two genera were found to be parasitizing SPWF in increasing numbers in Florida: Encarsia transvena, E. nigricephala, Encarsia (Aleurodiphilus) tabacivora, Eretmocers californicus and Encarsia near formosa. They report that E. transvena, E. californicus and E. formosa were the main parasitic wasps infesting heavy populations of SPWF recovered from field collection.

Encarsia transvena has been found in populations of papaya whitefly, Trialeurodes variabilis and in SPWF in Central Florida. Its development time is rather short, 12 to 14 days at 25°C, and is thus considerably less than that of the SPWF. This parasitoid is also very tolerant to high humidity levels (Hoelmer and Osborne 1990)

Encarsia formosa Gahan is the only species commercially available in Florida (Osborne et al. 1990). Host feeding behavior is similar to that of E. transvena, but it requires three more days at 25°C to complete its life cycle. Adult survival of E. formosa depends on low humidity and cooler climates. This parasitoid has been suggested as a management tactic for control of the SPWF (Hoelmer and Osborne 1990).

The papaya whitefly is being considered as a possible alternate host for natural enemies using the "banker-plant" distribution method. The banker-plant system consists of the distribution of natural enemies for SPWF control (Meeker 1990). For instance, the host-specific papaya whitefly would be used to disseminate parasitoids in the larval and pupal stage to SPWF populations without introducing additional unparasitized SPWF (Hoelmer and Osborne 1990). These researchers state that this distribution method could help in overcoming the reluctance of farmers to bring pests in with natural enemies, and would eliminate the need for isolating and repacking the parasitoids following production.

Encarsia lahorensis has been used successfully to control citrus whitefly, Dialeurodes citri populations on commercial citrus crops in Florida (McCoy 1985). E. lahorensis was introduced into the citrus-growing regions of Florida in fall 1977 and winter 1978. During the fall of 1978, the parasite established itself well in the Lakeland-Auburndale area, and has since spread throughout Florida (McCoy 1985).

Females of Eretmocerus californicus deposit eggs under all SPWF instars on the leaf surface but prefer mainly the second and third stages. The larva completes its development as an endoparasite in the host, requiring 18 to 24 days at 25°C. A number of predators have been identified attacking SPWF populations. Most of the predators mentioned include mites (Acarina: phytoseiidae), coccinellids (Coleoptera:

coccinellidae), and lacewings (Neuroptera: chrysopidae) (Gerling 1986). Amblyseius aleyrodis, A. swirskii, Typhlodromus medanicus and Euseius hibisci for phytoseiidae; Chrysoperla sp. for chrysopidae, and Coccinella septempunctata and Delphastus pusillus for coccinellidae (Gerling 1986, Hoelmer and Osborne 1990) are the most important predators recorded.

Predators are mobile during both the larval and adult stages and are also active at night, making it difficult to establish their role and value in a particular host. As a consequence, use of these predators as biocontrol agents against whiteflies has not yet been established. Meyerdirk and Coudriet (1985) in their study of predation of the phytoseiid Euseius hibisci on SPWF under laboratory conditions found that E. hibisci did not use SPWF as a primary food source in the field if other hosts were available. However, this predator was capable of completing development from a 1 day-old protonymph to adult while regulating the population density of whiteflies by feeding on a combination of eggs and first and second instars.

More recently, Hoelmer and Osborne (1990) reported that the most promising predator to use in greenhouses is the coccinellid, Delphastus pusillus Casey. They state that this beetle is distributed in the southern and eastern U.S., Caribbean, and Central and northeastern South America. Both larvae and adults feed on eggs, immature, and adult

whiteflies. Development time under greenhouse conditions is 21 to 22 days. Adult females live an average of 50 days while males about 40 days. This predator is best adapted to feeding and reproducing at high whitefly population densities.

An entomopathogenic fungus, Paecilomyces farinosus, attacking SPWF on cotton plants was reported in 1971 (Nene 1973). SPWF mortality caused by the fungus was more than 90%. Recently, pathogenic fungi are being studied as a potential measure for biological control programs against the SPWF in Florida. Studies conducted at the CFREC, UF/IFAS, Apopka, FL, by Osborne and Hoelmer (1990) demonstrated the virulence of Paecilomyces fumosoroseus and its possible role as biological control agent against the SPWF. Fourth instar larval of SPWF exposed to spore concentrations of 1×10^4 and greater (100% R.H.), showed substantial whitefly mortality within three days of post-inoculation. The percentage of whitefly killed also increased with time. Possibilities of the incorporation of these Paecilomyces into IPM programs for whiteflies in Florida are currently being investigated by the above authors.

The SPWF natural enemies described above can be valuable biological agents for control of whitefly populations under natural conditions. Most of the natural enemies against whiteflies have not been sufficiently studied to estimate their potential role in controlling the SPWF in agricultural situations (Gerling and Horowitz 1986). Most of the parasitoid and predator species are very susceptible to

insecticides. Bellows and Arakawa (1988) in studies of dynamics of the SPWF and Eretmocerus sp. in cotton fields found that percent parasitism of the nymphal population increased during the season in the 1982 and the 1983 sites. In both years, parasitism was low (less than 10%) until September, when it began to increase approximately coincident with the cessation of pesticide treatments. The increase in parasitism was slower in the 1983 populations than in the 1982 populations and that may have been related to the heavy pesticide usage in the 1983 commercial sites. Meyerdirk et al. (1986) found similar results with Encarsia sp., in which they showed high activity in late August, but their effectiveness against SPWF was decreased by additional pesticide applications. Natwick and Zalom (1984) found 70% parasitism of SPWF by mid-October in cotton plots not treated with pesticides.

Chemical Control

Chemical control has often been the main weapon against most pests of agriculture. This is because synthetic organic chemicals possess general reliability, rapid action, flexibility in meeting changing agronomic and ecological conditions, and the ability to maintain the high quality of agricultural products that is demanded by consumers (Metcalf 1982). Without chemical control, man's crops would be ravaged by diseases, insects and weeds, resulting in severe loss of food production (Matthews 1979). Development of new chemicals

has often been the focus in the approach to control major pests.

More than 56 insecticides from 7 chemical classes have been evaluated for their effectiveness against SPWF (Sharaf 1986). Adequate chemical control of SPWF is difficult due to the spatial distribution of the insect in the crop canopy. The preference of SPWF sessile stages (eggs, nymphs and pupae) for the underside of leaves in the lower part of the crop canopy not only protects them from extreme climate changes, but also from insecticide sprays applied from above the canopy (Matthews 1986). Usually direct contact by the insecticide is required, and this means that 95% control would require spraying the underside of leaves, which is an extremely difficult task (Stansly and Schuster 1990). Since whiteflies are sucking pests, only systemic insecticides will be ingested. Vydate is the only systemic insecticide registered for use on tomatoes (Price et al. 1988).

Sharaf (1986) reviewed the chemicals used to control the SPWF over the past decade. Overall, carbamates such as aldicarb, oxamyl, carbofuran, and carbaryl were the most effective pesticides. Synthetic pyrethroids including cypermethrin, fenvalerate, permethrin, and bifenthrin were generally very effective for SPWF control. Other chemicals such as chlorinated hydrocarbons (e.g. endrin, endosulfan, DDT, and lindane), diphenyl compounds (Sharaf 1986), bacterial fermentation products (e.g. abamectin), mineral and botanical

oils, and chitin synthesis inhibitors (Ishaaya et al. 1988) have also been reported. Price et al. (1989) identified chemical compounds permitted for use on ornamental crops (e.g. poinsettias) that provide an effective control of either adult or nymphal SPWF for greenhouse or field. They reported that under greenhouse conditions, lindane, endosulfan, and sulfotepp were effective against adults. Abamectin, bifenthrin, and permethrin were effective against nymphs and adults.

The effectiveness of insecticides on whiteflies depends greatly on their physiochemical and biological characteristics. The SPWF is a sucking pest and therefore, any insecticide that breaks down rapidly on the leaf surface and does not translocate within the plant would not be expected to be effective against whiteflies unless it hits them directly (Sharaf 1986). Insecticide effectiveness on SPWF also depends on the method and frequency of application of the insecticide, the spray volume rate, and dosages or amounts of active ingredients used for the insecticide (Sharaf 1986).

Insecticidal combinations and new insecticides to improve SPWF control were evaluated by Schuster et al. (1989). They studied the effects of the combination and alternation of current registered insecticides for use on Florida tomatoes and determined which ones produce $\geq 90\%$ mortality of at least one life stage of the whitefly. About 50 insecticidal

treatments were evaluated. They found significant mortality in the combination of methamidophos + permethrin on large nymphs and adults. Alternations of pyrethroids (permethrin, esfenvalerate), endosulfan, insecticidal soap and oxamyl were effective in managing SPWF. New insecticides or insecticides that had not been evaluated in the greenhouse and laboratory trials and that appeared effective in controlling the SPWF in the field trials included bifenthrin and the combination of endosulfan and parathion. Abamectin, alternated weekly with endosulfan, was effective in small and large plots under commercial conditions. The authors suggest that growers should take into consideration some factors when developing an insecticide program for their farms. For instance, growers should read the insecticide label thoroughly before selecting and applying any insecticide. The insecticide label is the law and insecticides cannot be used contrary to the label. Since all lifestages of the SPWF will probably be present in tomato fields, growers should select insecticides or insecticide combinations or alternations that kill adults and immatures. When insecticides are applied frequently, a grower may need to alternate among three or more different insecticides to avoid exceeding label restrictions.

Populations of whiteflies usually have overlapping generations. Because of this and the stage specificity of the insecticides, frequent and regular applications of insecticides are required. This process, however, might lead

to the build-up of resistant strains of whiteflies. This can happen when farmers and pesticide users assume that the whiteflies did not receive a lethal dose, and the farmer may react by increasing the pesticide dosage and frequency of application, which results in increased selection of resistant individuals.

Insecticide Resistance

Nature of Insecticide Resistance

Resistance was first observed in 1914 in the San Jose scale, Quadraspidiotus perniciosus (Comstock), selected by lime sulfur spray (Metcalf 1980). By 1946, a total of 11 species of insects were resistant to insecticides (Metcalf 1982). Among the insecticides reported causing resistance in these species were lead arsenate, sodium arsenite dip, potassium antimonyl tartrate, and cryolite.

The greatest increase and strongest impact of resistance to insecticides has occurred during the last 40 years, after the discovery and extensive use of synthetic organic insecticides and acaricides (Georghiou and Taylor 1986). More than 447 species of insects and mites have developed resistance to insecticides, and the most significant increases occurred in species of agricultural importance. Insecticide resistance began to receive the scientific attention deserved only after World War II when the newly developed "wonder" insecticide DDT failed to control resistant strains of the housefly, Musca domestica L., in Sweden and Denmark in 1946,

and the mosquitoes Culex pipiens L. in Italy, and Aedes sollicitans in Florida in 1947 (Metcalf 1982). Since then, with the proliferation of new insecticides and the widening scale of their employment, cases of insecticide resistance have continued to develop at an exponential rate.

Resistant strains develop through the survival and reproduction of pre-adaptive individuals carrying a genome altered by one or more possible mechanisms that allows survival after exposure to an insecticide (Brattsten et al. 1986). Moreover, most of our crop plants have natural chemical defensive components and toxins or allelochemicals (e.g. alkaloids, terpenes, and phenols) to repel or kill many of the organisms that attack them (e.g. insects and pathogens) (Brattsten et al. 1986). In turn, attacking organisms have evolved some mechanisms that enable them to detoxify or resist these defensive chemicals of their hosts. This ability of insects to metabolize and to degrade enzymatically these natural toxicants provided insects with the metabolic machinery to attack many insecticides and thus has contributed to the rapid development of resistance in some insects (Terriere 1984).

Mechanisms of Resistance

Mechanisms of resistance arise through inheritable changes in the genome of individual insects (Brattsten et al. 1986). The mutation of structural genes can result in a critical modification of the gene products such as a decreased

target size sensitivity or increased ability to metabolize pesticides (National Research Council 1986). According to Plapp (1986), two types of regulatory genes are of major importance in insecticide resistance and both differ in inheritance and biochemistry. One type exhibits all or none inheritance (fully dominant or recessive) and appears to involve changes in the amount of protein synthesized. The second shows codominant (intermediate) inheritance and involves changes in the nature of proteins synthesized. In addition to mutations that spread through selection, resistance to insecticides in insects is preadaptive. Terriere (1982) states that the genes controlling the resistance mechanisms are already present in the population and have been present prior to any use of man-made chemicals. This suggests that exposure to insecticides has (so far at least) caused no increase in the mutation rate.

The principal biochemical mechanisms of resistance in insects include 1) reduction in the sensitivity of target sites, 2) metabolic detoxication of the pesticide by enzymes such as microsomal oxidases (MFO system), glutathione-S transferases, and carboxyesterases, and 3) decreased penetration and/or translocation of the pesticide to the target site in the insect (Terriere 1984, National Research Council 1986). Plapp (1986) states that quantitative decrease in numbers of target sites may be involved in target-site resistance to DDT/pyrethroids and cyclodiens in the housefly.

Induction of different detoxifying enzymes is the key to metabolic resistance. Terriere (1982) reports that microsomal oxidases increase the metabolism of the toxicant, usually producing less toxic metabolites. Glutathione -S transferases are involved in the detoxication of some organophosphate insecticides (i.e. parathion and diazinon) in the housefly. Carboxylesterases appear to be responsible for most of the resistance now present in the peach potato aphid, Myzus persicae Sulzer in England (Devonshire and Moores 1982). In general, these enzymes have the ability to convert lipophilic foreign compounds (or xenobiotics) to polar metabolites that can be excreted and are more water soluble (Brattsten et al. 1986). These enzymes function through the hemoprotein cytochrome P-450 and they have the requirement of a typically high degree of substrate lipophilicity. The role of cytochrome P-450 in insects was first studied in the housefly Musca domestica (Wilkinson and Brattsten 1972).

According to Brattsten et al. (1986), the mechanisms of physiological resistance to toxic chemicals include diminished penetration, sequestration, and excretion. The rate of penetration depends on the physical characteristics of the molecule and on the properties of the insect integument which vary considerably between species and life stages. Sequestration of synthetic insecticides can be observed in the case of the peach potato aphid in which the esterase responsible for resistance has high binding affinity but low

catalytic reactivity. Thus, the esterase functions as a storage protein for carbamates, organophosphates and pyrethroids (Devonshire and Moores 1982).

As new insecticides become more difficult to discover, develop, register, and manufacture, it is important to create new strategies that would delay or minimize the likelihood of resistance evolution (Georghiou 1980). IPM and insecticide resistance management are now regarded as essential to accomplish these purposes.

Methods to detect Resistance to Insecticides

There are three methods for resistance detection and monitoring for pest species: the classical bioassay test, the biochemical test, and the immunological test (National Research Council 1986).

Classical bioassay techniques have been the base for resistance detection and monitoring methods (Brent 1986). Standardized bioassay methods for resistance determination have been developed by the Food and Agricultural Organization (FAO) (Anonymous 1979, Busvine 1980) and the World Health Organization (WHO) (1976). According to Leeper et al. (1986) these methods provide more effective means for detecting the frequency of a trait within a field population. Nevertheless, although standard laboratory bioassays detect the level of resistance to different insecticides, they are unable to identify or measure the level of the enzyme associated with resistance.

The most common techniques used to detect resistance are topical application, precision spray applications of standard solutions, and the residual film of contact insecticides (Busvine 1971, Metcalf 1982). The topical application technique has been adopted for standard tests of resistance in higher Diptera and ticks by WHO (1976) and for root maggot flies, rice stem borers, green peach aphids, rice leafhopper, and boll weevils by FAO (Anonymous 1979). Small droplets from pipettes and other apparatus are applied on the insect body.

The residual film technique has frequently been used for bioassay work and tests for insecticide resistance and for screening tests to evaluate chemicals as possible insecticides (Busvine 1971). For bioassays or resistance tests, the deposits of insecticides are usually applied in a standard volume of volatile solvent. The solvent evaporates to leave either pure crystalline (or liquid) insecticide or a solution of insecticide in a non-volatile oil. Various type of surfaces have been treated such as glass, metal, leaves, fresh paint, dried mud, and filter paper (Busvine 1971).

Residues are produced by dipping, spraying, or painting the substrate. In the dipping test, the formulated insecticide is simply applied by dipping either the whole plant or part of it in a formulation of the type to be used in practice. After drying, insects are put on them. For experiments using rather artificial media for resistance test, residues are often prepared by application of solutions in

volatile solvents. The residual insecticide is dissolved in a volatile solvent and spread evenly over a test surface. A wetting agent can be used to cause the solution to wet the surface and spread evenly. In another type of test, the insects are restricted to part of one surface of a simple leaf. This is done by confining the insects in a glass ring or in various types of plastic cells or cages. This method of exposing insects to sprayed leaves can be used to assess residual potency of foliage of plants weathered in the field. When the water dries from aqueous dip residues, a concentrated residue is left. The toxicity of this residue will depend to a considerable extent on its physical state. Under certain conditions, the penetration of a toxicant may depend on its crystallization from a colloidal deposit and on the solubility of the compound in the epicuticular wax of the insect (Busvine 1971).

Several dip-test bioassays have been used and described in the literature to detect resistance to insecticides in whiteflies. A bioassay method for measurement of insecticide resistance in immature stages of the whitefly, T. vaporariorum, has been described in the FAO Plant Protection Paper (Busvine 1980). Resistance studies using a dipping test on immature whiteflies have been reported by Wardlaw et al. (1973), Watve et al. (1977), and Nazer and Sharaf (1985). A method to detect insecticide resistance in adult whitefly was suggested by FAO (Busvine 1980). Documentation on insecticide

resistance in adult whiteflies are given by Watve et al. (1977), Wardlow et al. (1976) and Elhag and Horn (1983).

Residual film bioassays have also been used to detect insecticide resistance in the SPWF as reported by Toscano et al. (1984), Dittrich et al. (1985), Prabhaker et al. (1985), and Ahmed et al. (1987). Residual film bioassays have also been used with insecticides combined with synergists to detect toxicity levels in the SPWF (Horowitz et al. 1988a, 1988b, Prabhaker et al. 1988, and Dittrich et al. 1990b).

Biochemical tests identify the unique detoxication enzyme associated with resistant pests and have been reviewed by Sawicki et al. (1978) and Miyata (1983). Recently immunological tests for resistance have been based on identification of detoxification enzymes using monoclonal antibodies (Devonshire and Moores 1984).

Insecticide Resistance in the SPWF

Although the SPWF was known to occur on cotton for many years (Russell, 1975), it was not until the late 1970s that the SPWF was considered a primary cotton pest in Sudan (Dittrich et al. 1985). Insecticide resistance in the SPWF on Sudanese cotton was reported in the 1980s by Dittrich et al. (1985) and Ahmed et al. (1987). The SPWF became resistant to insecticides as a result of heavy aerial applications of DDT and other insecticides to control the American bollworm, Heliothis armigera (Hubner). The highest resistance ratios were found for dimethoate and monocrotophos as a consequence

of a long selection with the DDT/dimethoate combination introduced in 1964 (Dittrich et al. 1985). The SPWF adult was more susceptible than the nymph and the resistance ratio of the adults was generally higher than that of the nymphs (Ahmed et al. 1987). During the last decade, widespread use of pyrethroids on cotton for the control of the American bollworm has caused an upsurge of homopterous pests, among them the SPWF, in countries such as Turkey and Sudan. As a result, high levels of pyrethroid resistance have been detected (Dittrich et al. 1990a).

Resistance to synthetic pyrethroids by the SPWF in the U.S.A. was initially studied in California by Toscano et al. (1984) on lettuce. Results from bioassay tests indicated a 14-fold resistance to deltamethrin for the Imperial Valley strain, (brought in 1981), when compared to the laboratory strain at the LC_{50} level. Later, Prabhaker et al. (1985) demonstrated a broad spectrum of resistance to organophosphates and pyrethroids in the SPWF in southern California from three different field populations. Resistance to organophosphates (sulfos, methyl parathion, and malathion), DDT, pyrethroids (fenvalerate and permethrin), and carbamates were detected for the field strains indicating considerable heterogeneity in their response. This high level of resistance to various insecticides appeared to be due to the repeated exposure of the SPWF to all these chemicals which were used to control the cotton pest complex, including

whiteflies. Toscano et al. (1985) reported resistance to three pyrethroids (permethrin, cypermethrin, and fenvalerate), and two organophosphates (parathion and monocrotophos) in all the field populations collected in the Imperial Valley. Degrees of resistance in larvae, pupae, and adults in the SPWF were not equal. Later on, Prabhaker et al. (1989) found that the resistance ratios varied with each stage of the SPWF, and each stage was subject to different degrees of selection pressure. They suggest that this differentiation in chemical sensitivity in the SPWF could have implications for control programs.

Resistance to insecticides has also been reported in other whiteflies, such as in the greenhouse whitefly, T. vaporariorum West, in England, the Netherlands, and USA (Wardlow et al. 1976, Elhag and Horn 1983, 1984), and in the bandedwing whitefly, T. abutilonea Haldeman, (Watve et al. 1977).

One approach for understanding the mechanisms of insecticide resistance in the SPWF involves the use of synergists. In general, synergists have the ability to inhibit specific detoxification enzymes involved in the mechanisms responsible for resistance to the pesticide (National Research Council 1986). Combination of various classes of synergists with insecticides in laboratory bioassays allow the identification of most types of mechanisms of resistance based on differential mortalities (Prabhaker et

al. 1988). Horowitz et al. (1988b) found that S,S,S-tributyl phosphorotrithioate (DEF) synergized both cypermethrin and permethrin causing almost complete elimination of resistance in the resistant (R) strain of SPWF. That result suggested that esterases are involved in the pyrethroid resistance in the SPWF. Prabhaker et al. (1988) used selective synergists such as DEF, piperonyl butoxide (PB), and triphenyl phosphate (TPP) and determined that esterases were detoxifying three organophosphate (OP) compounds and one pyrethroid. They also found that TPP synergized malathion, which indicates the involvement of carboxyesterases in malathion resistance. Dittrich et al. (1990b) used the synergists PB and tricresylphosphate (TCP) to detect the presence of mixed-function oxidases (MFO) and nonspecific esterases, respectively, for four populations of SPWF. The bioassay technique adopted for this study was the leaf-dipping method developed by Dittrich et al. (1985). The presence of highly active nonspecific esterases in the populations from Guatemala and Nicaragua were detected with the application of monocrotophos plus the synergist TCP. Esterase activity for cypermethrin was high in the populations from Nicaragua, followed by those from Guatemala and Sudan. These esterases were revealed with the synergist PB. Indication of high mixed-function oxidase (MFO) activity was found in the populations from Guatemala and Nicaragua when monocrotophos were synergized by PB. Measurements of acetylcholinesterase

(AChE) sensitivity to inhibition by monocrotophos and carbofuran revealed that populations from Turkey, Sudan, and Guatemala were insensitive to monocrotophos. On the other hand, the population from Nicaragua was considerably more resistant to carbofuran than that of the other three populations.

Insecticide Resistance Management

Management Strategies

The problem of insecticide resistance has promoted the development of new strategies in attempts to manage resistance. However, few management strategies have been put into practice and one of the reasons is because of the different perspectives that individual groups may have on the problem and how it should be solved (Leeper et al. 1986).

Efforts to manage resistance fall into two major categories: 1) IPM strategies to minimize chemical use, and 2) chemical use strategies, which prescribe how chemicals should be used to minimize resistance development (Dennehy et al. 1987). In order to devise chemical usage strategies, it is essential to develop methods for monitoring insecticide resistance that would allow the detection and characterization of resistant pests (National Research Council 1986).

According to Metcalf (1982), the most fundamental approach to resistance management in IPM is to minimize the selection pressure that leads to resistance. This can be done by decreasing the frequency and extent of insecticide

applications and using insecticides only when damage is likely to exceed clearly defined economic thresholds (Metcalf 1980). The reduction of pesticide use not only decreases selection pressure on pest insects, but also preserves natural enemies and other non-target species, reduces environmental contamination, reduces the exposure of growers and consumers to potentially toxic materials, and may reduce phytotoxicity (Hammock and Soderlund 1986).

Another insecticide resistance management strategy includes not only the use of existing compounds, but also the discovery and development of new chemical control agents (Hammock and Soderlund 1986). However, new insecticides are introduced less frequently, and the cost of discovery and development has risen sharply along with the time required for the process (Metcalf 1980). New insecticides also need to be compatible with IPM programs and resistance management programs to extend their usefulness. Therefore, new strategies of resistance management are established at the very outset of the commercial life of a new chemical. Further monitoring and checking are done to know better if new chemicals are working properly or need to be modified or intensified (Brent 1986). New management strategies for specific resistance problems also need to provide agriculturists with effective, simple, reliable methods for determining resistance followed by a straight-forward protocol for responding to such conditions (Dennehy 1987).

One objective of resistance management is to maintain resistance alleles at very low frequency. Recommended management tactics should be aimed at reducing allele frequencies, reducing dominance, and minimizing the fitness of resistant genotypes (Leeper et al. 1986). Another indirect approach is to rotate (alternate) insecticides. This assumes that resistant genotypes have substantially lower fitness than the susceptibles (Georghiou 1980). One approach to decrease dominance of resistance is the immigration of susceptible individuals and low-resistance genes frequencies (Tabashnik and Croft 1982). Fitness reduction of resistant genotypes to susceptible genotypes can be done by either preserving susceptible homozygotes or eliminating heterozygotes and resistant homozygotes (Leeper et al. 1986). Other ways to lower fitness include reducing insecticide use rates, extending intervals between treatments, and using short residual insecticides. However, it is difficult to decide which tactic is more appropriate and will maintain effective control.

Insecticide Resistance Management in the SPWF

Since insecticide resistance in the SPWF was first reported in Sudanese cotton (Dittrich et al. 1985, Ahmed et al. 1987) and subsequently in the U.S. on cotton (Prabhaker et al. 1985), some research studies to manage the resistant phenomenon for the SPWF are being considered. Detection of insecticide resistance in the greenhouse whitefly (Elhag and

Horn 1983,1984) and in the SPWF (Prabhaker et al. 1985) demonstrated that high doses or frequent application and continuous lifetime exposure to insecticides hastened the intensification of insecticide resistance in these whiteflies. The urgency to moderate insecticide use to retard the evolution of resistance in whiteflies or in another pest led researchers to plan carefully the use of the insecticides in control programs or IPM programs.

According to Sawicki and Denholm (1987), the implementation of insecticide resistance management was strongly stimulated by the need to take steps to forestall or overcome resistance to the synthetic pyrethroids which had, when first introduced in the late 1970s, restored total control over multi-resistant pests in cotton. When the SPWF was reported to be resistant to synthetic pyrethroids in California (Prabhaker et al. 1985), and since it is a plant disease vector (Duffus and Flock 1982), tactics to control this pest and its resistance problem urge the need for developing resistance management tactics against the SPWF.

Managing insecticide resistance in whiteflies is rather a recent effort which demands more research. Emphasis needs to be directed toward laboratory and field evaluation of new strategies for preventing or slowing the development of resistance in whiteflies. Research on evaluating resistance management strategies for insecticide resistance management in the SPWF is being conducted in California due to the problem

of resistance in this area since 1985. One approach has been the augmentation of traditional insecticides with synergists such as DEF and PB to increase the toxicity of organophosphates and pyrethroids. Prabhaker et al. (1988) report the significant synergistic effect of DEF ($SR = 15.7$) with permethrin, causing a 15-fold increase in toxicity in one of the field strains tested from California. As a result, the level of resistance was reduced from 87 to 47-fold. A second method of attack has been directing insecticides against larval stages in addition to treatment of adults. Prabhaker et al. (1989) found that insecticide treatments were most effective during the first and second larval stages of a resistant strain of SPWF. However, there was a decreased insecticide susceptibility in the egg and pupal stages suggesting difficulties in insecticide management programs. On the other hand the adult stage of SPWF was resistant to insecticides. A third technique has been the use of natural chemicals with a unique mode of action, e. g. to reduce oviposition and egg viability, and the use of antifeedants or repellents against SPWF. All of these approaches are supported by resistance monitoring to manage resistance and to detect shifts in susceptibility within a population.

Monitoring Insecticide Resistance

It is essential to maintain regular surveillance over the susceptibility of populations of insect pests before prescribing specific insecticide treatments in IPM programs.

Several techniques for determining insecticide resistance are essential in insecticide resistance management programs. These monitoring methods attempt to measure changes in the frequency or degree of resistance in time and space, as well as to provide early warning of resistance. Resistance monitoring is most useful when undertaken early in a resistance episode. It can also be used to evaluate the effectiveness of alternative tactics that are employed to overcome, delay, or prevent the development of resistance (Brent 1986).

As explained in a previous section, bioassay tests have been widely used for monitoring resistance to insecticides. For instance, Keil et al. (1985) developed a topical application technique for establishing baseline susceptibility data for L. trifolii. This technique provided a reliable method for evaluating the toxicity of insecticides against this leafminer. The dose response lines of two strains of L. trifolii to permethrin revealed the development of resistance in one of the strains that was collected at a chrysanthemum range in San Diego, California, where standard insecticides had failed to control the leafminer. Mason and Johnson (1987) used a residue technique to acquire toxicity data that could be valuable for development of resistance management programs for L. trifolii. The development of new and improved standard methods to detect and monitor resistance to key pests is needed (National Research Council 1986).

Another important factor in an insecticide resistance management program involves the compilation and acquisition of quantitative data on susceptibility of key pests to various insecticides. Baseline data is obtained with a reference strain of known susceptibility from which it is possible to select a diagnostic concentration that may be used to monitor samples of the pest for resistance. A series of repeated bioassays are carried out in order to establish reliable base-line data for susceptible strains. These series of finding the best doses to kill 50% of pest are the framework of baseline data. Regression lines are normally obtained by plotting the percent mortality expressed in probits against the log-dose. This dose-response line is a regression line that may represent the baseline of susceptibility. From it can be read the best estimate of mortality at any doses of a particular insecticide. This baseline will help us to compare other dosage-mortality in which there are suspected episodes of resistance.

Some researchers consider that monitoring for resistance by comparing LD_{50} s and slopes between field populations is inefficient for detecting an incipient resistance outbreak (Roush and Miller 1986). They believe that a diagnostic dose (or discriminating dose) is more practical and reliable for monitoring resistance in the field, as it can detect resistant individuals when it is present at frequencies of $< 10\%$. (Sawicki et al. 1978, Dennehy et al. 1983).

Baselines of susceptibility have been found frequently in situations in which a pest population has already developed resistance to one or several insecticides. As a result of the high cost to get new insecticides, more studies in resistance are being conducted soon after an insecticide is put into the market (Dennehy 1987). The acquisition of quantitative data on base-line susceptibility of a pest forms part of insecticide resistance management program designed to prevent or delay development of resistance in pests.

CHAPTER 3

MATERIALS AND METHODS

Reference Colony

A colony of Bemisia tabaci Genn. was started during the fall of 1988 from an infestation on pansy, Viola tricolor, at the Central Florida Research and Education Center (CFREC), UF/IFAS, Apopka, Florida. This population had not been exposed to any insecticide pressure for at least 15 generations before toxicological tests were carried out. For the purpose of this study, this colony was considered as a susceptible reference colony. An efficacy test was conducted in a greenhouse using several insecticides at the commercial recommended rates. Results indicated a susceptible population. Material from this reference colony was identified by Dr. A. Hamon at the Department of Plant Industry, Gainesville, Florida, as B. tabaci Genn.

General Rearing Procedure

The reference colony in this study was reared on yellow crookneck squash (Cucurbita pepo L.). Squash seeds were planted periodically in a metro-mix® soil (growing medium, Grace Horticultural Products, W.R. and Co., Cambridge, Massachusetts, 02140) using plastic pots (13 cm diam. x 12 cm high) and grown in a room ($27 \pm 2^{\circ}\text{C}$ and 90% R.H.) separated

from the insect rearing room. The reference colony and plants were kept in metallic frame cages (60 x 60 x 60 cm). The bottom side was covered with an aluminum sheet while the two laterals, the rear and the top of each cage were covered with a fine nylon screening cloth (mesh size of 0.0064 cm²) to prevent adults from escaping. The upper 3/4 of the front was covered with transparent plexiglass attached to the frame with screws while the lower 1/4 was fitted with stockinette sleeving used to gain access into the cage. The caged plants and whiteflies were maintained in a rearing room at temperature of 27 ± 2°C, 83% RH and a 12:12 (L:D) photoperiod. Fresh plants were provided when needed for oviposition and feeding throughout the rearing. Plants were watered every other day to maintain the required water level.

The following procedure was conducted in order to standardize the age of SPWF adults for bioassays. Adults of unknown age were aspirated from the rearing room with a suction device consisting of a modified cordless rechargeable vacuum cleaner (Cohen et al. 1989) that was modified further to collect the insects directly into a cylindrical fonda carton container (Fonda ®). The fonda carton container had in the end facing the vacuum a piece of mesh cloth, while the other end had a lid with a hole (1 cm ID). Adults were then released into a metallic cage containing five uninfested potted squash plants that served to support the new population for feeding. Approximately 2000 adults were allowed to

oviposit for 24 h before removing the plants from the oviposition cage to another metallic cage free of SPWF adults. Adults emerged 19 ± 1 day after oviposition. Excessive watering was avoided to prevent a high humidity that might promote growth of fungi.

Insecticides

Insecticides used in this study were selected based on their use by Florida ornamental and vegetable growers. These insecticides were obtained from the following sources: The organochlorinated endosulfan (Thiodan 3 EC, FMC Corporation, Middle Port, NY); the pyrethroids cypermethrin (Cymbush), bifenthrin (Talstar 10 WP, FMC Co.), and fenvalerate (Pydrin 2.4 EC, E.I. Du Pont de Nemours and Co., Wilmington, DE); the microbial derivate abamectin (Avid 0.15 EC, Merck, Sharp and Dohme Co., Rahway, NJ); the organophosphates chlorpyrifos (Lorsban 4 EC, Dow Chemical Co. Midland, MI) and acephate (Orthene 75 S, Chevron Chemical Co., Richmond, Calif.); and the carbamate oxamyl (Vydate 2L, Du Pont de Nemours & Co.). The insecticides bioassayed as a leaf residue against SPWF populations in clip cages were formulated material. For sticky tapes, formulated or technical materials were used.

Reference Colony Characterization

A bioassay was carried out to test the efficacy of insecticides on the SPWF feeding upon tomato plants. Tomato seeds (cultivar Walter) were planted in a 96 cells tray (Com-Packs) containing metro-mix soil. At least three seeds per

cell were planted to secure emergence. After water was added to the soil, the tray was transferred to a growing room at $27 \pm 2^{\circ}\text{C}$ and 90% R.H.

When seedlings had emerged, they were transplanted to one gallon pots and moved to a greenhouse where they stayed until the study was over. The fungicide Ridomil 2E was applied at a rate of 0.2 ml/gal to control Pythium. In the greenhouse, the temperature fluctuated between 26°C at night and 32°C at noon. The average relative humidity was 90%. The greenhouse glass was coated with whitewash to minimize the amount of radiant energy entering the house which would increase the temperature inside the greenhouse causing water stress to plants but also might cause leaf burning. Bamboo stakes were placed in each pot to give support to plants during their growth stage. Tomato plants were infested 30 days after planting with whiteflies collected in the reference colony with the modified hand-held vacuum cleaner. The whiteflies were transported in the fonda carton adapted to the aspirator. A total of 10 fonda cartons with whiteflies were transferred to the greenhouse and then distributed on two benches containing the tomato plants. Adults were released from fonda cartons, allowing them to distribute randomly on plants.

Three insecticide applications were made at eight, sixteen, and twenty four days after the first infestation with SPWF. Three concentrations were used per insecticide (low, intermediate, and high). The intermediate concentration was

the dose rate recommended commercially in the field for each insecticide. The other two concentrations (low and high) were selected to observe if they had any significant effect on the population. Plants were sprayed with test solutions on their upper leaf surfaces to the point of spray run-off with a two gallon capacity plastic, hand compressed-air sprayer. This application was performed outside the greenhouse. When plants had dried, they were transferred back into the greenhouse and distributed randomly throughout. The sprayed tomato plants were reinfested by introduction of 16 pots with squash plants heavily infested with SPWF from the reference colony to the greenhouse. These plants were transported in cages (60 x 60 x 60 cm). Reading was made 48h after the first application. Only live adults were counted.

Bioassay Methodology

Leaf Residue. Henderson bush bean leaves (Phaseolus vulgaris L.) were used for the leaf residue bioassay. Plants were grown in pots (4 cm ID x 12 cm high) in the plant-growing room and were ready to use ten days later. Serial dilution was used to prepare the desired concentrations. For each insecticide concentration, the wetting agent X-77 (Chevron Chemical Co, Richmond, Calif.) (0.6 ml/l) was added to ensure a better dispersion of the insecticide on leaves. Leaves were dipped for six seconds in 500 ml beakers containing insecticide concentrations. Any excess liquid present on

leaves was shaken off. The leaves were allowed to dry for an hour.

Cages to hold SPWF on a treated leaf were made similar to those described by Kishaba et al. (1976) with some modifications, and are called clip cages in this work. Each clip cage consisted of two clear plastic rings (2 cm ID x 1 cm high) fused to a curl hair-clip. Foam rings were glued to one of the surfaces of each ring that was to make contact with the leaf. The ring that goes on the underside of the leaf had the same kind of cloth that was used to make the cages. It was glued onto the other surface, forming the cage body. A small hole (0.5 cm ID) was also made in this ring to introduce the adults. This hole was closed with a stopper made of cotton wrapped with the same material of the screen cloth. A regular metallic paper clip was used to support the clip cage to a bamboo stake using small rubber bands.

Adults were aspirated into each clip cage from the population of whiteflies to be tested with a hand-made aspirator. This mouth aspirator consisted of a modified pasteur pipette and a clear plastic tube. Between the modified pipette and the tube, 2 cm of kimwipes® (Kimberly-Clark Corporation, Roswell, GA 30076) paper and 2 cm of mesh were placed to hold aspirated insects in the pipette. The tip of the aspirator was fitted in the hole from the clip cage. Adults were forced to leave the aspirator by tapping on it with the fingers. No carbon dioxide for anesthesia was

necessary to transfer adults. At least 20 SPWF adults were confined in each clip cage that was clamped to each treated leaf with the cage on the underside of each leaf. Clip cages were carefully labelled with name of the treatment and number of replication. Mortality readings were made 24 h after exposure. Mortality was determined by counting through the ring of the clip cage the dead adults. When mortality was high, live adults were counted instead of the dead ones. In that case, mortality was determined by subtracting the live adults from the total. The total number of adults present in each cage was also obtained after freezing those adults that were still alive.

The dip-test bioassay was chosen for both techniques (clip cages and sticky tapes) because it has been the method most used to detect insecticide resistance in whiteflies (Wardlow and Ludlam 1976, Busvine 1980, Elhag and Horn 1984, Toscano et al. 1984, Prabhaker et al. 1985).

Sticky Tapes. The sticky tape technique was similar to the one described by Haynes et al. (1986). Sticky tapes were made by cutting 9 cm long pieces from a roll (183m long x 5 cm wide) of yellow plastic tape (polyethylene, 0.08 mm thick) (Olson Products, Inc.). This tape is sold with a sticky material called Sticky Stuff® to make a long sticky tape to trap insect pests in the field. The insect sticky material, Tree Tanglefoot, (Tanglefoot Company, Grand Rapids, MI) was used to make the tapes. Tree tanglefoot is a natural gum

resin product softened with castor oil used to prevent insects from climbing trees (Webb et al. 1985). The desired insecticide test concentrations were prepared by adding the required quantity of insecticide to 90 ml of hexane, and then addition of 10 ml of tanglefoot. Several concentrations of tanglefoot were tested, and 10% gave the best results. Less than 10% of tanglefoot allowed adults to fly away and escape, and more than 10% increased mortality because adults would be drowned in the tanglefoot. Once sticky concentrations were ready, properly labelled tapes were dipped for 6 seconds. Sticky tapes were then put on wet paper towels inside a plastic box (22.9 x 31.75 x 9.8 cm), to produce a high humidity necessary to reduce mortality in the control. However, the plastic box was not closed hermetically. A gap (0.02 cm) was left between the lid and the box, to allow air flow. This was done in order to prevent adult mortality as a consequence of the condensation that could occur due to the high humidity inside the box and the temperature of the growing room ($27 \pm 2^\circ \text{C}$). In this way, control mortality was $< 10\%$. Whiteflies were collected with the mouth aspirator and sprinkled onto the sticky tapes by taping gently with the fingers on the aspirator. Anesthesia was not needed to transfer adults from the colony to the tapes. Mortality was assessed 24 h. after dipping tapes, using a light microscope. The criteria for mortality was complete motionlessness. Whiteflies that were not moving were prodded with a very fine

metallic hair attached to a wood stick, to be sure that they were dead. For both of the above bioassays (leaf residue and sticky tape) a single bioassay had 4-5 concentrations, untreated check, and 5-6 replications each. At least 20 adults were used for each concentration dosage with a total sample size of 500 adults per bioassay. Formulated insecticides were used to treat tapes and leaves. Insecticides were mixed with hexane in the sticky tape bioassay and with tap water in the leaf residue bioassay.

Preliminary Range Finding Studies

Each of the insecticides tested was carried through the process of finding the appropriate concentration range to use in the bioassays. The starting dose for each insecticide was higher than that from the commercially recommended dose to control the SPWF. For the leaf residue bioassays, the dose was diluted in 500 ml of tap water. For sticky tape bioassays, the dose was diluted in 100 ml of tanglefoot (Tanglefoot Co., Grand Rapids, MI) + hexane. The new concentration was diluted in half and five to six dilutions in half were done until the first concentration range was found. Concentrations were expressed in mg of active ingredient [AI]/ml. The number of replications for the leaf residue bioassay was three. For the sticky tape bioassay, the number of replicates was increased to six.

Stability of Sticky Tapes over Time

One hundred and eighty tapes were dipped in a dilution of insecticide + hexane + tanglefoot as explained previously. The following treatment concentrations were prepared: abamectin 0.0072 mg [AI]/ml, bifenthrin 1.20 mg [AI]/ml, chlorpyrifos 0.96 mg [AI]/ml, endosulfan 0.359 mg [AI]/ml, and fenvalerate 0.720 mg [AI]/ml. These values were chosen based on the LC_{50} s found for those insecticides. The control was dipped in hexane-tanglefoot only. Each treatment was replicated six times. Tapes were laid down on paper towel in between two cafeteria trays (33 x 48 cm), one of them upside down. Tapes were stored in their own set of trays for each replicated insecticide, with a total of six sets of trays (one tray per treatment). Trays were kept at a room temperature of $25 \pm 2^\circ\text{C}$. Five dates were selected in a two-week period plus another one month after dipping. At each date, 30 tapes were used. They were placed in boxes containing wet paper towels as previously described. At least 20 SPWF adults from the reference colony of the same age and size were aspirated and sprinkled on tapes. The boxes were kept in a controlled environmental room at $27 \pm 1^\circ\text{C}$. Adult mortality was recorded 24 h. later under a binocular light microscope.

Susceptibility of the SPWF to Selected Insecticides based on their Age and Size

This study was conducted to determine if variability in preliminary results of leaf residue and sticky tape bioassays

might be due to differential mortality in adults of different age and size. SPWF adults have sexual dimorphism in which females are larger than males (Lopez-Avila 1986a). Therefore, since adults used in bioassays were picked up by eye, it was more convenient to work with their size rather than their sex. Two tests were made using the leaf residue and sticky tapes methodologies, respectively.

The test with the leaf residue methodology was made with formulated endosulfan at the concentrations of 0.09 and 0.18 mg [AI]/ml. The latter concentration was obtained from preliminary tests with clip cages in which LC_{50} for endosulfan (Thiodan 3EC) was 0.18 mg [AI]/ml. The first concentration was chosen in order to test the toxicity effect at lower concentration. This treatment was replicated three times. SPWF adults from one to seven days old were utilized. Adult age was standardized by rearing SPWF populations in metallic cages as it was previously explained. The cages were set every three days because the number of adults that emerge from SPWF nymphs in each cage can be used in two or three bioassays. SPWF adults from one to six days old were used. Every day one age of the adults was tested during six consecutive days.

The second test with the sticky tapes methodology was also made considering age and size of adults and only one concentration of the five selected insecticides. The following were the insecticide concentrations prepared:

endosulfan 0.36 mg [AI]/ml, abamectin 0.0072 mg [AI]/ml, chlorpyrifos 0.48 mg [AI]/ml, fenvalerate 0.72 mg [AI]/ml, and bifenthrin 1.2 mg [AI]/ml. These concentration were chosen from the LC_{50} values obtained in preliminary bioassays. The sticky tapes used in this study were dipped in the same day. They were maintained in kitchen trays at a room temperature of 25 ± 2 °C. Each tape was divided by a line in two sectors in order to locate large and small adults in the same tape. Twenty SPWF adults were put in each sector approximately. Although adults of the SPWF may live more than six days, they were only considered from one to six days old in this study. Adult mortality was counted 24 h after locating them on the tape.

Toxicity of Selected Insecticides to the Laboratory Reference Strain and Florida Field-collected Strains of SPWF

Baselines of susceptibility were determined using the bioassay techniques of leaf residue and sticky tapes. Preliminary bioassays were carried out to find the dose-range of susceptibility to selected insecticides. Large, two day old whitefly adults were used for this purpose.

Field populations of SPWF were kindly provided by Dr. D.J. Schuster (GCREC, UF/IFAS, Bradenton, FL); Dr. J.E. Peña and Dr. R. Jansson (TREC, UF/IFAS, Homestead, FL); Dr. P. Stansly (SWFREC, UF/IFAS, Immokalee, FL) and Dr. Locascio (Department of Vegetables, UF, Gainesville, FL).

All field populations were collected from plants that were heavily infested with the SPWF. Although leaves had different stages of the SPWF, those having mainly pupae were preferentially selected to reduce mortality in the mailing process. Field populations from Bradenton, Homestead, and Immokalee came from infested tomato, Lycopersicon esculentum. The Gainesville population came from infested squash, Cucurbita pepo, cantalope, Cucumis melo reticulatus, and watermelon, Citrullus lanatus. These crops were known to have been exposed to continuous applications of insecticides. Each population was placed in individual metallic cages, properly labelled (name, date), and kept in a separate room (27 ± 2 °C, 83% RH and a 12:12 [L:D] photoperiod). Uninfested squash plants (about 10 days old) were provided as food for the emerging adults. When pupal population was high, part of it was put in the refrigerator to help slow down pupal development, and to make pupae available for several bioassays. Emerged adults were used when they were one or two days old. Adults of the same age from the reference colony were also exposed to the same treatments.

Baseline susceptible data for selected insecticides was obtained with both bioassay methods. However, determination of toxicity to insecticides from the four Florida SPWF populations was only done with the sticky tape bioassay. The following were the insecticide concentrations prepared to test those populations: endosulfan 0.18, 0.36, 0.72 and 1.44 mg

[AI]/ml, chlorpyrifos 0.48, 0.96, 1.92 and 3.84 mg [AI]/ml, and fenvalerate 0.72, 1.44, 2.88 and 5.72 mg [AI]/ml. These concentrations were selected from those used to find the baselines on sticky tapes.

Statistical Procedures

Data were subjected to probit analysis with the POLO-PC program (Le Ora Software 1987), the personal computer version of the POLO (Probit or Logit) program developed in 1977 for use on large mainframe computers. POLO-PC performs the computations for probit or logit analysis with grouped data (Finney 1971). Probit analysis was used to determine the toxicity of selected insecticides to the laboratory strain and field-collected strains of SPWF. The probit analysis provided the intercept and slope of the regression line for each strain. The analysis of variance (ANOVA) from data of characterization of the reference colony test was made with MSTAT program (Michigan State University, 1986). Means were separated in order to be compared against the control only. Data were transformed to $\sqrt{(X+0.5)}$ and $(\text{LOG} + 1)$ before performing the ANOVA. $(\text{LOG} + 1)$ was selected for presenting lower coefficients of variance.

Analysis of variance of data for the effect of age and size of SPWF adults to endosulfan was made using SAS program (SAS Inst. 1985). Results obtained from probit analysis were corrected for outliers as described by Preisler (1988).

For determination of susceptibility response of the SPWF to selected insecticides based on their age and size with the sticky tape bioassay, data were statistically analyzed to obtain mortality percentages and by an analysis of variance using a SAS program (SAS Inst. 1985). For the sticky tape bioassay, corrected percent mortality data was plotted using Lotus-Freelance Plus program (Lotus Development Co. 1989). This program provided regression lines and their equations and correlations. The corrections were made using Abbott's formula (Abbott 1925), as follows:

$$\text{Corrected \% mortality} = \frac{\text{Test \% mort.} - \text{Contr. \% mort.}}{100 - \text{contr. \% mort.}} \times 100$$

This formula assumes that deaths from handling and from the insecticide are independent and uncorrelated. Data from stability of sticky tapes over time was statistically analyzed by obtaining the percentages of each treatment unit and corrected with Abbott's formula. The ANOVA was performed using SAS.

The percent mortality data for toxicity of selected insecticides to the laboratory reference strain and field-collected strains of SPWF was also corrected with Abbott's formula. The level of resistance of a field population was calculated using the resistance ratio (RR). It is the LD_{50} of the resistant population over the LD_{50} of the normal susceptible population (Metcalf 1982).

CHAPTER 4

RESULTS AND DISCUSSION

Susceptibility of Reference Colony

The results from evaluating the susceptibility level of the SPWF used as a reference strain in bioassays is shown in Table 1. This strain was considered to be a potential reference strain based on the fact that it was free of insecticide pressure for more than fifteen generations. The results from treatments with seven insecticides at three different concentrations indicated that this was a susceptible strain. The middle concentration for each insecticide corresponded to the commercial recommended rate to control SPWF on vegetable and/or ornamental crops.

Endosulfan, oxamyl, fenvalerate, bifenthrin, and abamectin insecticides, which are registered for SPWF control on tomatoes (Schuster et al. 1989) and on flower crops (Price et al. 1989) in Florida, were used to determine the susceptibility of the reference colony. Acephate and chlorpyrifos were also chosen for study because the former has been evaluated in greenhouse and laboratory trials and appeared effective in controlling the SPWF on tomato (Schuster et al. 1989), while chlorpyrifos has been recommended for control of many pests including whiteflies (Thomson 1982).

Table 1. Insecticide efficacy bioassay with SPWF on tomato plants with commercially recommended concentrations.

Insecticide	Concentration (Mg [AI]/ml)	Adults alive*
Endosulfan	0.144	40.50 bc
	0.288	28.00 bc
	0.359	10.00 c
Fenvalerate	0.144	3.00 c
	0.431	1.25 c
	0.719	0.00 c
Bifenthrin	0.050	1.50 c
	0.100	3.50 c
	0.200	0.00 c
Abamectin	0.00072	0.50 c
	0.00180	0.00 c
	0.00900	0.25 c
Acephate	0.375	91.25 b
	0.750	18.25 c
	1.500	19.00 c
Chlorpyrifos	0.488	0.00 c
	1.067	2.75 c
	1.221	0.00 c
Oxamyl	0.480	25.75 bc
	0.960	10.25 c
	1.920	0.00 c
Control	0	282.75 a
	0	261.00 a
	0	240.75 a

Means within columns followed by the same letter are not significantly different ($P < 0.05$; Duncan's multiple range test [SAS Institute 1985]).

* Adults present on four leaves forty-eight hours after insecticide application.

Furthermore, chlorpyrifos was registered to control SPWF on tomatoes in Florida in 1990. Stansly and Schuster (1990) in their evaluation of field trials at the SWREC (Immokalee, FL) in the fall of 1989 compared chlorpyrifos alone and mixed with esfenvalerate to control SPWF on tomatoes. They found few pupae with chlorpyrifos alone while with the mixture there were few nymphs. All of the insecticides tested were efficacious against SPWF adults. The results indicated that the reference colony was a susceptible strain of the SPWF.

Bioassay Development

Leaf Residue. The first results shown in this section are part of the preliminary concentration range finding. Finding the range of the concentrations during the initial testing was done in order to increase accuracy and to check degree of susceptibility to insecticides as suggested by Brent (1986). Acephate, chlorpyrifos, cypermethrin, fenvalerate, bifenthrin, endosulfan, and abamectin were tested. Acephate produced very low mortality of the SPWF adults at all concentrations. The results with acephate were inconsistent and high variability occurred between replications. The leaf residue bioassay may not have been suitable for assay of acephate. Table 2 shows the only bioassay that could be analyzed by probit analysis, and the LC_{50} found for acephate was higher than the concentration commercially recommended for the control of SPWF.

Table 3 shows the results of two SPWF populations treated with cypermethrin. One sample came from a commercial greenhouse (C.G.) population and the other sample came from the reference colony. The data could not be analyzed by the probit program. Increasing the concentration of cypermethrin did cause an increase in mortality of the reference colony SPWF but not in the greenhouse colony sample. Possibly the leaf residue bioassay also is not suitable to measure the effect of cypermethrin on SPWF. Adult mortality was low and the variability between replications was also high. These results differ from those of a previous report by Dittrich et al. (1990), in which control of SPWF adults with cypermethrin was detected with a residual film bioassay. A suitable response of cypermethrin with a residual test was also reported by Nazer and Sharaf (1985) on SPWF nymphs. When the SPWF adults were released in the clip cages attached to treated leaves, they showed a tendency to stay away from the leaf surface treated with cypermethrin. Cypermethrin may have a repellent effect upon SPWF adults. A further factor that may have contributed to variability was that standardization of age and size of adults was not considered at this stage of the study. Furthermore, the number of concentrations was three or four, and more replications possibly were needed for this kind of bioassay in which a large number of SPWF adults are necessary to obtain reliable data. The avoidance behavior of the SPWF adults when tested with cypermethrin was

Table 2. Toxicity of acephate to SPWF adults using the leaf residue bioassay.

Population	N	LC ₅₀ * (95% CL)	Slope $\pm$ SE
Reference	417	5.24 (4.6 - 6.5)	7.03 $\pm$ 1.1

N Number of SPWF adults used in the bioassay.

* Mg [AI]/ml.

Table 3. Toxicity of cypermethrin to SPWF adults using the leaf residue bioassay.

Concn.*	Reference population			Commercial greenhouse		
	N	Dead	SD	N	Dead	SD
0.0	24	1	1.0	19	3	1.1
0.014	22	1	1.1	23	2	2.9
0.028	23	3	1.7	20	1	1.5
0.072	27	3	3.5	23	1	1.2
0.144	28	5	1.5	22	1	2.8
0.288	23	5	1.9	20	1	1.7

N Number of SPWF adults per bioassay
(mean of three replications).

* Mg [AI]/ml.

unexpected. Visual observations showed that the adults became irritated after they were confined in the clip cages and moved away from the leaf surface to the clip cage walls. This behavior may be due to a repellent effect. Ruscoe (1977) observed the "avoidance reaction" of the adult weevil, Anthonomus grandis Boh., after contact with plants treated with permethrin. He interpreted this behavior as a repellent/antifeedant effect. The repellency of the pyrethroids has been noted by Burden (1975).

Chlorpyrifos was used in bioassays with the C.G. population and the reference population of the SPWF (Table 4). Mortality was similar in both populations. The confidence limits of the LC_{50} values overlapped and the difference between the slope values were relatively small. Bioassays Nos. 2 and 3 presented LC_{50} s twice higher than bioassay No. 1 made with the C.G. and reference populations. This difference might be attributed to the fact that bioassays Nos. 2 and 3 were made with SPWF adults of known age and size and six replications per bioassay. The slope values with chlorpyrifos were steeper than with the other selected insecticides. These values were similar for both the C.G. population and the reference population. It appears that both populations were genetically homogeneous and susceptible. They were easily controlled at the commercial recommended concentrations. Determination of the levels of toxicity by organophosphates to SPWF on cotton plants with a leaf residue bioassay was reported by Prabhaker

Table 4. Toxicity of chlorpyrifos to two adult populations of the SPWF using the leaf residue bioassay.

Population	#	N	LC ₅₀ * (95% CL)	Slope $\pm$ SE
C.G.▼	1	382	0.39 (0.28 - 0.49)	3.47 $\pm$ 0.3
Reference	1	428	0.33 (0.24 - 0.41)	3.97 $\pm$ 0.4
	2	574	0.68 (0.61 - 0.76)	7.37 $\pm$ 0.9
	3	419	0.57 (0.37 - 0.76)	4.74 $\pm$ 0.4

Bioassay number.

N Number of adults used in the bioassay.

* Mg [AI]/ml.

▼ Commercial greenhouse.

et al. (1985). They found low levels of resistance but with slope values for chlorpyrifos ranging from 2.14 to 3.17, indicating more genetic heterogeneity of their three SPWF strains relative to the strains tested herein.

The most reproducible results with the leaf residue method were found in bioassays of adult SPWF treated with endosulfan (Table 5). The first bioassay was made with adults of the C.G. population and the reference population. In two subsequent bioassays, only the reference population was used. The genetic heterogeneity indicated by the slopes was similar for both populations. The mean LC_{50} value for the reference colony was about four times greater than that of the commercial greenhouse colony, and this may mean that the reference colony was more resistant than the commercial one. Dittrich et al. (1985), in a dipping test using discs of cotton leaves treated with endosulfan and exposed to SPWF found a LC_{50} value of 1.6 ppm and slope value of 3.5 for the reference strain.

The susceptibility response of the SPWF adults to the toxic action of the pyrethroids bifenthrin and fenvalerate was surprisingly similar (Table 6). The LC_{50} values were practically the same based on the overlap of their confidence limits. The slopes were low indicating that the reference population is heterogeneous. The small differences found between the bioassays performed with these insecticides suggested compatibility with the leaf residue method.

Table 5. Toxicity of endosulfan to two adult populations of the SPWF using the leaf residue bioassay.

Population	#	N	LC ₅₀ * (95% CL)	Slope ± SE
C.G.▼	1	322	0.04 (0.03 - 0.05)	2.18 ± 0.3
Reference	1	438	0.13 (0.11 - 0.16)	2.71 ± 0.3
	2	733	0.12 (0.06 - 0.16)	2.28 ± 0.2
	3	438	0.13 (0.11 - 0.16)	2.71 ± 0.3

Bioassay number.

N Number of SPWF adults used per bioassay.

* Mg [AI]/ml.

▼ Commercial greenhouse

Table 6. Toxicity of bifenthrin and fenvalerate to insecticide susceptible SPWF adults in a leaf residue bioassay.

Population	#	Insecticide	N	LC ₅₀ * (95% CL)	Slope ± SE
Reference	1	Bifenthrin	516	0.13 (0.08-0.22)	0.95 ± 0.1
	2	Bifenthrin	230	0.10 (0.07-0.13)	1.22 ± 0.1
	3	Fenvalerate	339	0.17 (0.04-0.45)	0.74 ± 0.1
	4	Fenvalerate	493	0.15 (0.12-0.19)	1.29 ± 0.1

Bioassay number.

N Number of SPWF adults used per bioassay

* Mg [AI]/ml.

Prabhaker et al. (1985) found a low slope value of 1.23 with fenvalerate in a leaf residue bioassay using cotton leaves. This slope is similar to the slope values found here using similar residual method and Henderson bush bean leaves.

The last insecticide selected in this project was abamectin, and the results with this insecticide were highly reproducible (Table 7). Although the bioassays had different slopes, the differences were small. The relatively low slope values indicate genetic heterogeneity of the SPWF adults to abamectin. These bioassays were made with different generations of the SPWF, and the generations had some effect in the variability observed among bioassays. The effectiveness of abamectin on other pests (i.e. leafminers) using the leaf residue bioassay have been reported by Leibee (1988) and Parella et al. (1988).

There were some advantages in using the leaf residue bioassay in these toxicological studies. The leaf residue bioassay is similar to the actual application of an insecticide in the field, and adult mortality could be determined easily by visual inspection. This technique also had some disadvantages. It was necessary to use plants of the right size to hold the clip cages, and although the material used was inexpensive and unsophisticated, to set up a bioassay was time consuming and demanded a lot of space. It would not be practical to use in the field for monitoring resistance of the SPWF.

Table 7. Toxicity of abamectin to insecticide-susceptible SPWF adults with the leaf residue method.

Population #	N	LC ₅₀ *	(95% CL)	Slope ± SE
Reference 1	716	0.00012	(0.00010-0.00014)	3.19 ± 0.3
2	308	0.00027	(0.00024-0.00031)	3.32 ± 0.4
3	638	0.00022	(0.00020-0.00030)	2.59 ± 0.2
4	533	0.00038	(0.00028-0.00046)	2.28 ± 0.2
5	782	0.00034	(0.00027-0.00042)	3.34 ± 0.2
6	477@	0.00024	(0.00021-0.00028)	4.94 ± 0.5
7	505@	0.00023	(0.00013-0.00031)	1.93 ± 0.3

Bioassay number.

N Number of SPWF adults used per bioassay.

* Mg [AI]/ml.

@ These bioassays were separated in large and small adults. The marked numbers are the large adults used per bioassay. The unmarked numbers represent bioassays made with adults of random size.

Early tests of the insecticides repeatedly showed high variability in LC_{50} and slope values, and there was low reproducibility between bioassays. The lack of reproducibility was attributed either to lack of standardization of the size and age of the insect or to incompatibility of the method and the insecticide in question. The size and age of SPWF adults were considered in some of the bioassays. The results found in those bioassays were sometimes different from those found in bioassays with adults of unknown age and size. For instance, with endosulfan, bifenthrin, and fenvalerate there were no differences, but differences were found with chlorpyrifos and abamectin. In general, it was anticipated that all the insecticides were not going to be compatible with the leaf residue method, and this was one of the reasons to consider the sticky tape method as an alternative bioassay.

Sticky tape. The bioassays with this method were usually made with five concentrations and six replicates. The first results obtained with sticky tapes had a mortality in the control above ten percent. However, when the relative humidity surrounding the SPWF adults was increased, the mortality fluctuated between five and ten percent. The insecticides used were as follows: chlorpyrifos, endosulfan, bifenthrin, fenvalerate, and abamectin.

Table 8 shows the results of a probit analysis of dosage-mortality data for SPWF treated with chlorpyrifos. They

Table 8. Toxicity of chlorpyrifos to insecticide-susceptible SPWF adults with the sticky tape method.

Population #	N	LC ₅₀ *	(95% CL)	Slope ± SE
Reference 1	1088	0.29	(0.230 - 0.340)	5.10 ± 0.5
2	594	0.69	(0.502 - 0.810)	4.31 ± 0.7
3	453	0.84	(0.698 - 0.978)	4.29 ± 0.4
4	487	0.76	(0.608 - 0.913)	3.95 ± 0.4

Bioassay number.

N Number of SPWF adults per bioassay.

* Mg [AI]/ml.

present two important features. First, the LC_{50} values for the reference population and their confidence limits were reproducible, indicating that chlorpyrifos is compatible with the sticky tape method. Compatibility of chlorpyrifos with a similar sticky card method on L. trifolii adults has been reported by Haynes et al. (1986). He reported a LC_{50} value of 1.0 $\mu\text{g}/\text{mg}$ for a reference colony reared on chrysanthemums. Second, slope values were not only close suggesting reproducibility, but also they were relatively steeper than those values observed for other selective insecticides. This indicates that the SPWF population had a genetically homogeneous response to this insecticide.

Bioassays with endosulfan were tested with formulated and technical grade material (Table 9). The results found with this insecticide were highly reproducible, suggesting compatibility of endosulfan with the sticky tape method. Bioassays with formulated and technical grade endosulfan provided similar LC_{50} and slope values. Moreover, slopes values with this method were not different from those found with the leaf residue method.

The results with bifenthrin were similar to those found with fenvalerate (Table 10). The reaction response of SPWF adults to these pyrethroids on sticky tapes was similar to the response with the leaf residue method. The confidence limits of the LC_{50} values overlapped, indicating that both insecticide tests were reproducible on sticky tapes and compatible with

Table 9. Toxicity of endosulfan to SPWF adults of the reference population using the sticky tape bioassay.

Insecticide grade	#	N	LC ₅₀ * (95% CL)	Slope ± SE
Formulated	1	735	0.40 (0.34-0.47)	2.40 ± 0.2
	2	753	0.37 (0.30-0.45)	1.61 ± 0.2
Technical	3	794	0.44 (0.38-0.50)	2.57 ± 0.2
	4	720	0.39 (0.30-0.47)	2.57 ± 0.2

Bioassay number.

N Number of SPWF adults used per bioassay.

* Mg [AI]/ml.

Table 10. Toxicity of bifenthrin and fenvalerate to insecticide susceptible SPWF adults with a sticky tape bioassay.

Insecticide	#	N	LC ₅₀ * (95% CL)	Slope ± SE
Bifenthrin	1	745	1.53 (1.32-1.76)	1.81 ± 0.1
Fenvalerate	1	913	1.95 (1.60-2.41)	1.19 ± 0.1
Fenvalerate	2	991	1.47 (0.51-2.35)	1.96 ± 0.2
Fenvalerate	3	714	1.57 (1.37-1.81)	1.96 ± 0.1

Bioassay number.

N Number of SPWF adults used per bioassay.

* Mg [AI]/ml.

this method. The slopes obtained with this method were also low, indicating heterogeneity of the SPWF reference colony.

Abamectin was the last insecticide tested on sticky tapes. The bioassays performed with this insecticide showed high variability, suggesting incompatibility with the sticky tape method (Table 11). The LC_{50} s were different and the confident limits did not overlap. The results from the bioassays were improved when the humidity was increased in the treatments. According to Busvine (1971) humidity controls the amount of moisture sorbed on the surface in which the insecticide is applied. Water vapor appears to retard the sorption of insecticide or to displace the insecticide already sorbed. This would result in a greater mobility of the insecticide. The higher variability in tests with tapes compared with those from the leaf residue method may be due to a photodegradation process that leads to a rapid dissipation of abamectin. Light could have changed the chemistry of the insecticide to metabolites less toxic to the insect (Wislocki et al. 1989). A number of authors have noted abamectin ability to provide long residual control of spider mites on cotton, despite its rapid photodecomposition following application. This persistence is due to the translaminar action of abamectin, which has been observed in both laboratory and field bioassays with spider mites (Wislocki et al. 1984). Sticky tapes presented a number of advantages. They provided continuous contact of SPWF with the residual

Table 11. Toxicity of abamectin to insecticide-susceptible SPWF adults with a sticky tape method.

Population #	N	LC ₅₀ *	(95% CL)	Slope ± SE
Reference	1 382@	0.00193	(0.00150-0.00237)	4.00 ± 0.5
	2 851@	0.00720	(0.00297-0.00986)	3.10 ± 0.3
	3 592	0.00110	(0.00059-0.00170)	1.77 ± 0.2
	4 697	0.01600	(0.01000-0.02300)	1.60 ± 0.2

N Number of SPWF adults used per bioassay.

* Mg [AI]/ml.

@ These bioassays were separated in large and small adults. The marked numbers are the large adults used per bioassay. The unmarked numbers represent bioassays made with adults of random size.

film of the toxicant. They took the least amount of effort and tapes could be cut to a suitable size for the bioassays. Finally, they can be used to monitor resistance of the SPWF in the field. They also had some disadvantages. Once the adults are introduced to the tape, they had to be maintained in a high relative humidity to minimize mortality apparently due to desiccation. In addition, adult mortality had to be counted with a dissecting scope by a trained person.

Stability of Sticky Tapes over Time

The time that insecticides can remain on sticky tapes will influence the use of sticky tapes for monitoring resistance in the field. The results from ANOVA of mortality vs time did not show significant differences for the interaction insecticide x age (Table 12). In this analysis data for day 9 were deleted upon advice of Dr. John Cornell, statistician, IFAS Gainesville, as an outlier. The analysis indicates that the age of tapes did not influence the effectiveness of the insecticides during the fifteen days studied.

The percent mortality on day nine was very high for all insecticides. Since this happened with all tapes, it appears that the whiteflies used in day nine tests may have been of poor quality. An analysis of variance was made, however, with day nine data included, and the interaction of insecticide x age was still not significant ($F=0.86$, $d.f.=4,149$, $p=0.4874$).

Table 12. Analysis of variance for an insecticide persistence bioassay tested with SPWF adults on sticky tapes.

SOURCE	DF	SS	F	P > F
Model	9	1.4522	5.23	0.0001 *
Error	115	3.5485		
Corrected total	124	5.0007		

R-Square	CV	Root MSE	Y Mean
0.29	18.27	0.17	0.09

SOURCE	DF	SS	F	P > F
Insecticide (I)	4	0.5236	4.24	0.0031 *
Age of tape	1	0.7536	24.42	0.0001 *
I x Age of tape	4	0.2569	2.08	0.0877 NS

* Data preceded by this symbol are significantly different at ($P < 0.05$).

NS Non-significant.

The variability in this experiment was probably not caused by chemical changes in the insecticides on the tapes, but rather by variability in physiological responses of the SPWF adults to the insecticides. Variability in physiological response is supported by the fact that similar variability patterns were found for all five insecticides, and in the control, although it was less obvious in the control. The mean mortality response when tapes were one day old was between 78% and 85%, and when tapes were fifteen days old the mortality was between 50% and 70%. Overall the results suggest that insecticides may be persistent enough to make tapes useful over a 2-weeks period in the field, but more tests should be done in which SPWF populations are more uniform to control that source of variability.

It is important to mention that insecticide concentrations were based on LC_{50} values on sticky tapes. For monitoring resistance some researchers suggest (Busvine 1980, Roush and Miller 1986) working with a diagnostic concentration, which might mean increasing the LC_{50} value by five or ten fold in order to eliminate all susceptibles. Although the analysis of variance showed significant differences among the ages of the tapes for all five insecticides, those differences may not reflect changes on the tapes themselves, but may be caused by variability in the insects.

Effects of Age and Size on Susceptibility Response of the SPWF to Selected Insecticides

The effects of age and size on the response of SPWF adults to insecticides were evaluated with the leaf residue and sticky tape bioassays. The first test was made with endosulfan at two concentrations using the leaf residue bioassay. Table 13, shows the means of the percent mortality of SPWF adults.

Table 13. Corrected means of the percent mortality of large and small adults of SPWF exposed to two concentrations of endosulfan.

SPWF age (days)	0.09 mg [ai]/ml		0.18 mg [ai]/ml	
	Large	Small	Large	Small
1	19.0	68.0	84.7	83.2
2	15.0	54.3	50.0	29.6
3	81.4	66.7	90.0	76.8
4	71.1	48.3	56.0	76.8
5	44.9	83.1	60.0	89.2
6	0	62.2	82.6	40.2
7	71.0	77.9	77.0	82.0

The analysis of variance (ANOVA) for this test was made by selecting the three variables, size, age, and concentration, as well as their interactions (Table 14). Significant effects were found for all the sources of variation, with concentration showing the highest level of significance. Because of this, that analysis was separated by concentrations (Table 15). Age of adult SPWF, size and their interaction had significant effects on toxicity for the low concentration of

Table 14. Analysis of variance of the effect of age and size on mortality of SPWF adults with endosulfan at 0.09 mg and 0.18 mg [AI]/ml in a leaf residue bioassay.

Source of variation	DF	SS	F	P > F
Size	1	0.367	6.46	0.014 *
Age	6	1.072	3.14	0.010 *
Size * Age	6	0.989	2.90	0.016 *
Concentration	1	7.417	130.47	0.0001 *
Size * Conc.	1	0.319	5.62	0.021 *
Age * Conc.	6	0.931	2.73	0.021 *
Size * Age * Conc.	6	0.927	2.72	0.022 *

* Data preceded by this symbol are significantly different at ($P < 0.05$).

Table 15. Analysis of variance of the effect of age and size on mortality of SPWF adults with endosulfan at 0.09 mg and 0.18 mg [AI]/ml in a leaf residue bioassay.

Concentration	Source	DF	SS	F	P > F
0.09	Size	1	0.686	6.230	0.019 *
	Age	6	11.890	2.860	0.027 *
	Size * Age	6	1.879	2.840	0.027 *
0.18	Size	1	0.004	0.020	0.881 NS
	Age	6	1.952	2.050	0.093 NS
	Size * Age	6	1.460	1.530	0.205 NS

* Data preceded by this symbol are significantly different at ($P < 0.05$).

NS Non-significant.

0.09 mg [AI]/ml but not for 0.18 mg [AI]/ml. Results showed that size and age of SPWF should be considered in bioassays.

The second test was performed with endosulfan, chlorpyrifos, abamectin, bifenthrin, and fenvalerate using the sticky tape method. The ANOVA of percent mortality against age of SPWF adults showed a non-significant difference for the triple interaction insecticide x age x size (Table 16). The data indicated that the interaction age x size was not different among the insecticides. In this analysis two interactions, insecticide x age and age x size, were significantly different. The insecticide x age interaction demonstrated that the slopes of the lines were different among the insecticides (Figure 1). In the second interaction, age x size, the difference in the percent mortality between large and small SPWF adults was related with the age of adults (Figure 2). The results also demonstrated that small adults are generally more susceptible than large adults. This test was made to decide if it was necessary to select the size of adults in bioassays, and because of the results, large SPWF were selected for subsequent tests.

The toxicity of selected insecticides to SPWF adults was analyzed based on the test method (leaf residue or sticky tape) and the size of adults (Table 17). The LC_{50} values for large adults were higher than those for small adults in both methodologies. Only treatments made with bifenthrin in leaf residue bioassay and abamectin on sticky tape bioassay showed

Table 16. Analysis of variance for mortality data of small and large adults of the SPWF treated with selected insecticides.

SOURCE	DF	SS	F	P > F
Model	59	55973.46	6.46	0.0001 *
Error	216	31726.35		
Corrected total	275	87699.81		
R-Square	CV	Root MSE	Y Mean	
0.638239	15.13	12.12	80.12	
SOURCE	DF	SS	F	P > F
Insecticide (I)	4	25514.79	43.43	0.0001 *
Age	5	8413.10014	11.46	0.0001 *
I x Age	20	4868.84301	1.66	0.0423 *
Size	1	9337.56652	63.57	0.0001 *
I x Size	4	1161.10900	1.98	0.0992 NS
Age x Size	5	3048.36878	4.15	0.0013 *
I x Age x Size	20	1980.71665	0.67	0.8493 NS

* Data preceded by this symbol are significantly different at ($P < 0.05$).

NS Non-significant.

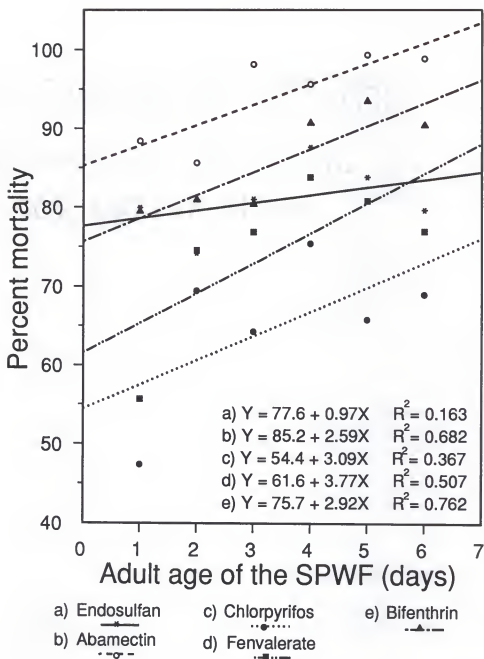


Fig. 1. Mortality response of SPWF adults treated with five selected insecticides based on adult age. Five replicates of approximately 25 SPWF adults per replicate were exposed to treated sticky tapes at six different ages of adults. Adult mortality was counted 24 h after each exposure.

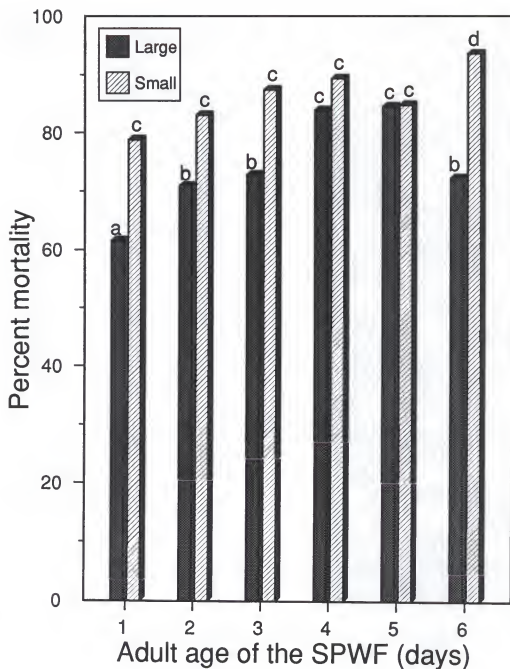


Fig. 2. Interaction between size and age of the SPWF adults treated with endosulfan, abamectin, chlorpyrifos, fenvalerate and bifenthrin. The interaction was similar for all treatments. Bars with the same letter at the top were not significantly different at ($P < 0.5$).

Table 17. Toxicity of selected insecticides to SPWF adults based on their size and the method used.

Insecticide	Method	Size	LC ₅₀ *	(95% CL)	Slope ± SE
Endosulfan	L. residue	L▲	0.124	(0.034 - 0.203)	2.32 ± 0.2
		S●	0.036	(0.019 - 0.049)	3.10 ± 0.3
	Sticky tape	L	0.223	(0.071 - 0.313)	3.25 ± 0.6
		S	0.159	(0.027 - 0.262)	2.36 ± 0.5
Chlorpyrifos	L. residue	L	0.575	(0.368 - 0.759)	4.74 ± 0.4
		S	0.402	(0.219 - 0.419)	3.58 ± 0.4
	Sticky tape	L	0.840	(0.698 - 0.978)	4.29 ± 0.4
		S	0.577	(0.381 - 0.731)	3.58 ± 0.4
Bifenthrin	L. residue	L	0.104	(0.075 - 0.134)	1.22 ± 0.4
		S	0.019	(0.004 - 0.041)	1.94 ± 0.3
	Sticky tape	L	1.460	(1.145 - 1.732)	2.38 ± 0.4
		S	0.908	(0.563 - 1.173)	2.31 ± 0.2
Abamectin▼	L. residue	L	0.232	(0.131 - 0.313)	1.93 ± 0.3
		S	0.162	(0.070 - 0.242)	1.83 ± 0.3
	Sticky tape	L	7.200	(2.970 - 9.860)	3.10 ± 0.3
		S	0.207	(0.170 - 0.240)	3.77 ± 0.5

▲ Large adults of the SPWF

● Small adults of the SPWF

* Concentration in mg [AI]/ml.

▼ Concn. in mg [AI]/ml x 10⁻³.

results with confident limits that did not overlap. The indication was that in most cases, the difference between both sizes was not significant. Therefore, either size could be used in bioassays when dealing with insecticide rates above the LC_{50} except for bifenthrin and abamectin in which it is necessary to consider the methodology as well. It is not known yet if the size of the adults could have significant effect at low rates as it was found for endosulfan. Standardization of age and size of SPWF adults seems to prevent any additional error besides that expected statistically.

Toxicity of Selected Insecticides to Susceptible Reference Strain and Florida Field-collected Strains of SPWF

Dose-Response Lines of the Reference Strain

Establishment of the baseline susceptibility data was obtained from the laboratory reference strain using both bioassay methods. The LC_{50} and slope values (Table 18) found for the insecticides studied with the leaf residue bioassays were smaller than those found with the sticky tape bioassays. An exception was found for chlorpyrifos with the leaf residue method. This insecticide showed a steeper slope than the rest of insecticides, which indicated that the population had a more homogenous response to chlorpyrifos than the other insecticides. The low slope in abamectin, fenvalerate, bifenthrin, and endosulfan with the leaf residue method not only indicates their genetic heterogeneity but also their high potential to develop resistance.

Table 18. Baselines of susceptibility of the SPWF adults to selected insecticides.

Insecticide	Method	LC ₅₀ *	(95% CL)	slope ± SE
Endosulfan	L. residue	0.124	0.034 - 0.203	2.32 ± 0.2
	Sticky tape	0.223	0.071 - 0.313	2.36 ± 0.5
Chlorpyrifos	L. residue	0.575	0.368 - 0.759	4.74 ± 0.4
	Sticky tape	0.840	0.698 - 0.978	4.29 ± 0.4
Fenvalerate	L. residue	0.153	0.117 - 0.195	1.29 ± 0.1
	Sticky tape	0.874	0.620 - 1.099	2.24 ± 0.2
Bifenthrin	L. residue	0.104	0.075 - 0.134	1.22 ± 0.4
	Sticky tape	1.460	1.145 - 1.732	2.38 ± 0.4
Abamectin	L. residue	0.0002	0.0001- 0.0003	1.93 ± 0.3
	Sticky tape	0.0072	0.0030- 0.0100	3.10 ± 0.3

* Concentration in mg [AI]/ml.

Results found with chlorpyrifos and fenvalerate were compared with those in California presented by Prabhaker et al. (1985), who also used a similar leaf residue test methodology. Although the LC_{50} values found with chlorpyrifos are surprisingly similar to those from Prabhaker, the slope values were quite different (4.74 ± 0.4 from 2.14 ± 0.29). Differences in toxicity levels for chlorpyrifos in this study when compared with those from California might have been due to the differences in host plants. While Henderson bush bean leaves were used in this study, Prabhaker et al. (1985) used cotton leaves in their bioassays. The influence of the host plant (Berry et al. 1980) on the level of toxicity of insecticides may explain at least some of the differences between these results and those of Prabhaker et al. (1985). On the other hand, slope values for fenvalerate from the reference colony in this study were similar to those obtained by Prabhaker. However, the LC_{50} for fenvalerate was different in both reference strains.

Although there is a difference between these two methods, the results showed consistency in their values. It is important to choose the method that better adapts to the insecticides in study. Data were corrected for outliers as suggested by Preisler (1988). He explained that there are five important types of departure from the probit-binomial model that produce large x values and that can be examined by residual plots. One type of model departure occurs when the

model fits all but few outlying values. An outlier will tend to have a large influence on the results, especially if it is observed at a response level near 0% or 100%. Outliers may convey important information, such as an interaction with an independent variable omitted from the model. Thus, an outlier should be discarded only if strong evidence suggests an error in recording or calculation or a similar type of circumstances. A second departure from the model occurs when one or several important explanatory variables that may affect the response have been omitted from the model, e.g. insect weight. In a third departure, the probit curve simply does not fit the data. Error terms that are not independent, e.g. insects from the same generation of a species might be more alike than insects from different generations, can cause a fourth type of departure. Finally, the error terms may not be binomial. In cases when unexplained sources of variability are observed between replicates, a model with a random effect fact can be fitted. The random effect could be attributed to the fact that subjects in the same replicate are in the same environment and possibly competing for the same resources. The dose-response lines of caged SPWF adults treated with selected insecticides are presented in Figure 3, and Figure 4 shows the results from applying the same insecticides to sticky tapes. They represent the dose-response values to be used for resistant management programs of the SPWF. The lines were drawn in a log scale. The values shown in the x-axis,

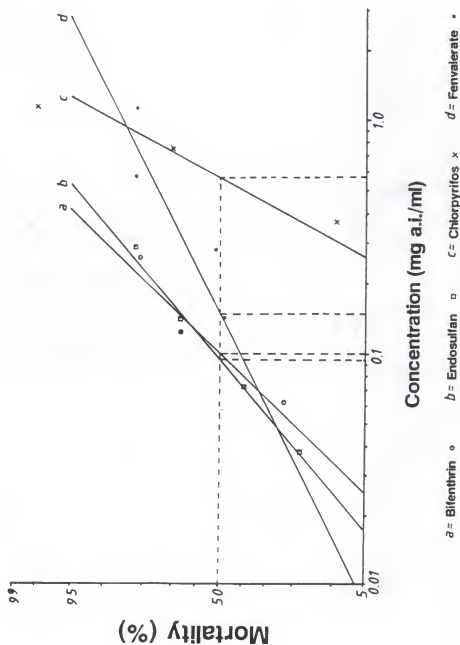


Figure 3. Baselines of susceptibility to selected insecticides of caged adults of the SPWF.

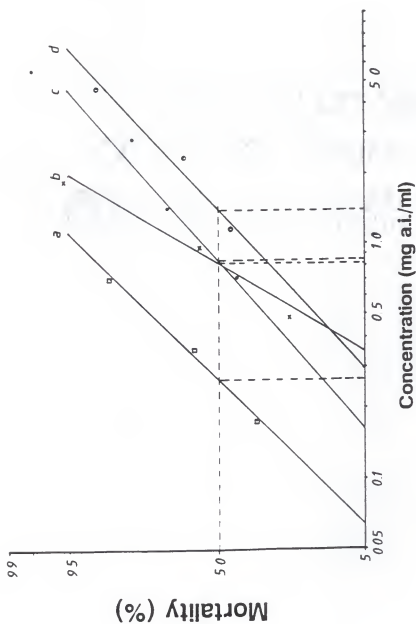


Figure 4. Baselines of susceptibility of the SPWF to selective insecticides incorporated in sticky tapes

however, are the true values in mg [AI]/ml. The regression equations were made with the slopes and LC_{50} values provided by probit analysis. The LC_{50} and slope values from field populations of the SPWF will be compared with the values observed in these baselines.

Toxicity of Insecticides to Florida Field-Collected Strains

SPWF populations from Bradenton, Gainesville, Immokalee, and Sanford, Florida, were collected during the spring and beginning of summer, 1990. These populations were obtained from field crops in which insecticides were applied periodically and where it was believed that they might be developing resistance. Results in Table 19 indicate that endosulfan was significantly more toxic to the Gainesville population than to the other populations. The population from Immokalee, was the least susceptible, but it was not significantly different than the Sanford reference colony. The slope and the LC_{50} values were higher than from the other three populations, suggesting the presence of a higher selective pressure. Although the LC_{50} values were not significantly different, they suggest that insecticide resistance may be developing in the Immokalee population.

Endosulfan has been used in south Florida in combination and alternation with other insecticides (such as permethrin and fenvalerate) for SPWF management (Schuster et al. 1989). It is too early to tell if the susceptibility that is present

Table 19. Toxicity of endosulfan to Florida populations of the SPWF on insecticide treated sticky tapes.

Population	N	LC ₅₀ * (95% CL)	Slope ± SE	RR
Bradenton	475	0.27 (0.20 - 0.33)	2.25 ± 0.2	1.2
Gainesville	507	0.08 (0.03 - 0.11)	2.37 ± 0.5	0.3
Immokalee	386	0.39 (0.27 - 0.48)	3.82 ± 0.5	1.7
Sanford▼	408	0.23 (0.06 - 0.34)	2.05 ± 0.4	1.0

N Number of SPWF adults used per bioassay.

* Concentration in mg [AI]/ml.

RR Resistance ratio.

▼ Reference population of the SPWF.

in those southern populations is due to management programs or to the fact that whitefly outbreaks only started a few years ago, chemical control was directed toward other pests and insecticides were not applied frequently. Dittrich and Ernst (1983) reported that the levels of resistance of the SPWF to endosulfan in Sudan were relatively low (21 X), but that endosulfan caused an increase in the insect fertility. Ahmed et al. (1987) found that the level of resistance of B. tabaci to endosulfan had increased 364 X after using this insecticide for about 18 years.

The population from Gainesville treated with chlorpyrifos was more susceptible than the population from Immokalee (Table 20). This broad spectrum insecticide has been recommended in ornamental and vegetable crops. The use of chlorpyrifos has been restricted in some vegetables, but it was permitted on tomato in 1990. The low exposure of the whiteflies to chlorpyrifos explains the level of susceptibility to it. Even in California Prabhaker et al. (1985) found a very limited resistance to chlorpyrifos. In three strains tested they found resistance ratios of 1.6, 2.6 and 3.9.

Fenvalerate is also a broad spectrum insecticide that has been widely used to control whiteflies and other pests in vegetables and ornamental crops. Table 21 shows the LC 50 values found for four Florida populations tested. The Homestead population was the most susceptible to fenvalerate, while the one from Gainesville was least affected. Low

Table 20. Toxicity of chlorpyrifos to Florida populations of the SPWF on insecticide treated sticky tapes.

Population	N	LC ₅₀ * (95% CL)	Slope $\pm$ SE	RR
Gainesville	576	0.67 (0.59 - 0.75)	4.10 $\pm$ 0.4	0.8
Immokalee	463	0.91 (0.81 - 1.00)	5.40 $\pm$ 0.7	1.0
Sanford▼	478	0.87 (0.79 - 0.95)	5.40 $\pm$ 0.5	1.0

N Number of SPWF adults used per bioassay.

* Concentration in mg [AI]/ml.

RR Resistance ratio.

▼ Reference population of the SPWF.

Table 21. Toxicity of fenvalerate to Florida populations of the SPWF on insecticide treated sticky tapes.

Population	N	LC ₅₀ * (95% CL)	Slope $\pm$ SE	RR
Gainesville	586	0.89 (0.67 - 1.09)	1.95 $\pm$ 0.2	1.6
Homestead	512	0.28 (0.08 - 0.48)	1.60 $\pm$ 0.3	0.5
Immokalee	487	0.65 (0.30 - 0.90)	3.00 $\pm$ 0.4	1.2
Sanford▼	499	0.55 (0.30 - 0.80)	2.56 $\pm$ 0.3	1.0

N Number of SPWF used per bioassay.

* Concentration in mg [AI]/ml.

RR Resistance ratio.

▼ Reference population of the SPWF.

susceptibility in the Gainesville population to fenvalerate was expected because repeated applications of this insecticide were made in order to control heavy infestations of the SPWF on vegetables. The slope value for the Gainesville population is low, suggesting that there is a genetically heterogeneous population that has more potential to develop resistance.

CHAPTER 5

CONCLUSIONS

The sweetpotato whitefly (SWPF) is a serious threat to the Florida vegetable and ornamental industry. Growers have experienced economically damaging outbreaks by the SPWF and the associated appearance of irregular ripening on tomatoes. The desire to control this pest and its transmitted virus diseases has led growers to rely heavily on insecticides, and therefore, there are more possibilities for SPWF to develop insecticide resistance.

Because the resistance status of a population can change rapidly, the development of new improved standard methods to detect and monitor resistance in the SPWF are needed. These monitoring methods are essential to early warning of resistance as well as in establishing the extent and severity of resistance.

In this research two methods, a leaf residue and a sticky tape bioassay, for estimating dose-mortality response of susceptible SPWF were developed. The leaf residue method consisted of exposing SPWF adults on treated leaves by confining the insect in a clip cage. In the sticky tape method SPWF adults were adhered to sticky tapes in which the toxicant was incorporated into the sticking agent. Both

methods required a dipping procedure in which the leaf or sticky tape was dipped into a solution of the desired concentration of insecticide prior to contact with SPWF.

The leaf residue method has some advantages over the sticky tape bioassay. The leaf residue bioassay is similar to the actual application of an insecticide in the field and may be preferred in situations where the efficacy of the insecticide is to be determined prior to use in the field. Clip cages to hold the insects on a treated leaf are also useful tools for residual film bioassays. Results from the leaf residue bioassay show that commercially recommended insecticides are still effective on the SPWF in Florida. Cypermethrin gave poor results with this method, but the leaf residue method can help to determine the dosage-response lines of SPWF adults to selected insecticides. Although the leaf residue method is tedious and time consuming to perform, it may be important as a tool to monitor changes in susceptibility to insecticides in the field. A review of the use of leaf residual tests in the literature supports the usefulness and importance of this test for determining dose-mortality response to insecticides in the SPWF.

The sticky tape bioassay was a modification of the common sticky traps currently used for monitoring and sampling whiteflies. It clearly would be a convenient advantage if the investigator or grower could prepare sticky tapes containing insecticides and use them as needed over a period of days or

weeks. The results from this study of age of tapes and mortality of SPWF over a period of 2 weeks do not give a clear cut indication that storage of tapes for 2 weeks is entirely satisfactory. The variability in mortality on different days after tape preparation was very high, and the predictability of regression lines (mortality vs. tape age) was poor. The high variability is believed to be primarily due to high variability in the SPWF populations used in tests. Until more bioassays are made to check on insecticide stability over time, it is recommended to prepare tapes the same day in which a bioassay is to be performed in field populations. Sticky tapes presented the following advantages: 1) they provided continuous contact of whiteflies with the residual film of the toxicant, 2) they are more suitable for field applications than the leaf residue bioassay and therefore more likely to be implemented in a monitoring insecticide resistance program, 3) they required less than half the labor needed by the leaf residue method and proved less difficult to perform, 4) they are easily adaptable to different situations because the tapes can be cut and shaped or provided with a rigid substrate to facilitate different sampling schemes in the field, 5) the problem of repellency that might be encountered in the leaf residue bioassay is overcome in the sticky tape bioassay, and 6) sticky tapes with the incorporated insecticide can be stored for more than two weeks before they lose half of their toxic effectiveness. One drawback to the sticky tape method

is that once the adults are introduced to the tape, the tapes need to be maintained in a high humidity environment to minimize mortality apparently due to desiccation. If the tapes were loaded with whiteflies in the field, special arrangements would have to be made in the field or laboratory to hold the sticky tapes for reading.

Both bioassay methods described here can be used for determining the dosage-response lines of commercial insecticides used to control the SPWF in Florida. Results from these two methods also provided reproducible LC_{50} s of the SPWF. With sticky tapes, LC_{50} s are between four and ten-fold higher than with leaf residue bioassays depending on the kind of insecticide used. The sticky tape technique appears to have wide applicability. Although similar sticky cards have recently been used for monitoring insecticide resistance in other pests, there are no reported data relative to using sticky cards or tapes in bioassays for determining insecticide resistance in the SPWF. The results and interpretations of the studies on toxicity of commercial insecticides using both bioassay methods are discussed in light of the problem of the SPWF management in vegetable and ornamental crops in Florida.

The size and age of whitefly adults seemed to play an important role in their capability to resist the toxic action of the insecticides. This condition, however, did not appear to be a significant factor for SPWF adults treated with endosulfan at high concentrations. Significant differences

due to size were found when endosulfan was used at low rates. At higher concentrations the differences due to size were not significant. Similar results were found with the other insecticides. There was not a good correlation between age of adults and percent mortality. From the results of the effects of size and age on insecticide susceptibility, it is suggested that large adults 2 days old should be selected in bioassays. The sex ratio is normally 2:1 in favor of females, which generally are larger than males. Males are more easily killed during handling than females, do not live as long as the females in the adult stage, and they are more susceptible to drowning in the sticky material of the tapes.

Determination of possible insecticide resistance levels in some Florida populations was evaluated with the sticky tape bioassay. Significant variation among field populations was observed, but Florida SPWF populations currently appear to be susceptible to recommended commercial insecticides. The slope values of the dose-mortality lines for the insecticides tested with the sticky tape method give an indication of the population homogeneity of SWPF. Although there was a low susceptibility level to endosulfan ($RR = 1.7$) from the Immokalee population, and to fenvalerate ($RR = 1.6$) from the Gainesville population, it was not considered highly significant because of the low resistance ratios. These low resistance ratios do not imply that resistance will not

increase, but rather that it has not occurred to a significant degree in SPWF adults in Florida.

Considered in conjunction with grower's reports of reduced effectiveness of these insecticides in some areas, the bioassay data presented here suggest that the observed low susceptibility to fenvalerate and endosulfan need further consideration when implementing monitoring strategies within an IPM program to detect early episodes of resistance by the SPWF. In addition, it is suggested that the sticky tape method described in this study can be used to obtain more field data from other areas in Florida in which suspected insecticide resistance is reported. In the longer term, an IPM program in Florida that incorporates resistance management programs with biological or cultural control must be established for better control of the SPWF.

Without doubt, more studies on insecticide resistance in the SPWF in Florida are needed due to an urgent demand to prevent and predict problems of insecticide resistance, which already has become a major problem in other areas in the United States.

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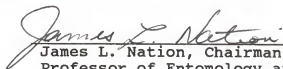
BIOGRAPHICAL SKETCH

Carlos Eduardo Mantilla Gonzalez was born in January 6, 1952, in Cali, Colombia. He received his high school degree in 1971 from Colegio Villegas in Cali, Colombia. He lived in Belgium from 1971 to 1974 where he conducted some undergraduate studies in physical sciences and agronomy at the Liege and Louvain universities. Facing financial struggles, he went back to his country to continue his career in agronomy.

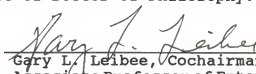
In 1975 he enrolled at the Universidad Nacional de Colombia, Facultad de Agronomia in Palmira, and received the Bachelor of Science degree with a major in agronomy (agricultural engineer) in 1981. Shortly thereafter, he started working for CIAT (Centro Internacional de Agricultura Tropical), Cali, Colombia, as a research assistant in the bean entomology program for 2 years. In 1983 he was awarded a grant from the British Council to pursue a Master of Science degree in applied entomology at the University of Newcastle upon Tyne, England. He graduated in 1985. He is currently a candidate for the degree of Doctor of Philosophy in the Department of Entomology and Nematology at the University of Florida, under the direction of Dr. Gary L. Leibe.

He married Miss Melba L. Moreno in 1983. He was a member of the Sociedad Colombiana de Entomologia from 1981 to 1985. He is currently a member of Entomological Society of America and Florida Entomological Society.

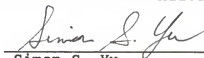
I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.


James L. Nation, Chairman
Professor of Entomology and
Nematology

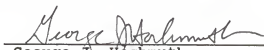
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Gary L. Leiber, Cochairman
Associate Professor of Entomology
and Nematology

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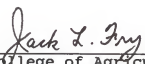

Simon S. Yu
Professor of Entomology and
Nematology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.


George D. Hochmuth
Associate Professor of
Horticultural Science

This dissertation was submitted to the Graduate Faculty of the College of Agriculture and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

August 1991



Dean, College of Agriculture

Dean, Graduate School